

# Supporting Information:

## Synthesis of Fluorogenic Polymers for Visualizing Cellular Internalization

Shane L. Mangold, Rachael T. Carpenter, and Laura L. Kiessling

*Departments of Chemistry and Biochemistry, University of Wisconsin-Madison, Madison-Wisconsin 53706*

*kiessling@chem.wisc.edu*

### General Procedures and Materials:

Rhodamine 110 (sold as Rhodamine 560) was purchased from Exciton (Dayton, OH). The ruthenium carbene  $(\text{H}_2\text{IMes})(3\text{-Br-py})_2\text{Cl}_2\text{Ru}=\text{CHPh}$  was synthesized as described previously.<sup>1</sup> Diethyl ether ( $\text{Et}_2\text{O}$ ) and tetrahydrofuran (THF) were distilled from sodium/benzophenone. Diisopropylethylamine (DIEA) and dichloromethane (DCM) were distilled from calcium hydride. Dimethylformamide (DMF) was rendered amine-free by treatment with Dowex 50Wx8-200 cation exchange resin,  $\text{H}^+$  form, 1g/L. Dimethylsulfoxide (DMSO) was stored over 3 Å molecular sieves. Distilled, deionized (dd or milliQ) water and 1000 molecular weight cut off (MWCO) dialysis tubing (Spectrum Laboratories) was used for the polymer purification unless otherwise noted.

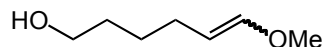
Analytical thin layer chromatography (TLC) was carried out on E. Merck (Darmstadt) TLC plates pre-coated with silica gel 60 F254 (250  $\mu\text{m}$  layer thickness). Analyte visualization was accomplished using a UV lamp, and charring with at least one of the following solutions: phosphomolybdic acid stain (Aldrich, diluted 1:1 with absolute ethanol), ceric ammonium molybdate, ninhydrin stain, or phosphomolybdic acid. Flash chromatography was performed on Scientific Adsorbents Incorporated silica gel (32-63  $\mu\text{m}$ , 60 Å pore size) using distilled reagent grade hexanes and ACS grade ethyl acetate ( $\text{EtOAc}$ ) or methanol and chloroform.

$^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC-300 or Varian Inova-500 spectrometers, and chemical shifts are reported relative to tetramethylsilane or residual solvent peaks in parts per million ( $\text{CHCl}_3$ :  $^1\text{H}$ :  $\delta$  7.26,  $^{13}\text{C}$ :  $\delta$  77.0; DMSO:  $^1\text{H}$ :  $\delta$  2.50,  $^{13}\text{C}$ : 39.5. Peak multiplicity is reported as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), doublet of triplets (dt), etc. The degree of polymerization (DP) was determined based upon integration of the phenyl protons relative to the polymer alkene protons. IR measurements were recorded on a Bruker Tensor 27 equipped with ATR and recorded as thin film. High resolution electrospray ionization mass spectra (HRESI-MS) were obtained on a Micromass LCT. Room temperature SEC was performed using THF as an eluent (1.0 mL/min) to determine  $M_w$ ,  $M_n$ , and polydispersity index ( $M_w/M_n$ ) values using a Viscotek GPCmax VE 2001 Solvent/Sample module equipped with a Viscotek TDA 302 triple detector array and two Polymer Laboratories Polypore 300X7.5 mm columns in series. Columns were calibrated with 10 narrow polystyrene standards (Polymer Laboratories EasiCal Polystyrene Standards (PS-1)) and data reduction was performed with OmniSEC software Version 4.5

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<sup>1</sup> Love, J.A.; Morgan, J.P.; Trnka, T.M.; Grubbs, R.H. *Angew. Chem. Int. Ed.* **2002**, 41, 4035-4037.

### (E,Z)-6-Methoxy-5-hexen-1-ol (1)



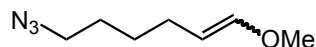
3,4-Dihydropyran (5.0 mL, 55 mmol) was cooled in an ice bath, and a 0.20 M aqueous HCl solution (21 mL) was added. The mixture was stirred on ice for 20 min and allowed to warm to room temperature with constant stirring for 45 min. The aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 x 20 mL). The combined organic layers were washed with aqueous saturated NaHCO<sub>3</sub> (2 x 20 mL) and dried over MgSO<sub>4</sub>. After concentration under reduced pressure, a colorless oil was obtained (4.18 g, 87%).

(Methoxymethyl)triphenylphosphonium chloride (3.00 g, 8.75 mmol) was dissolved in anhydrous THF (30 mL) and cooled to -78 °C. A solution of 0.50 M KHMDS in toluene (19 mL, 9.5 mmol) was added dropwise to the mixture. After addition, the reaction mixture was stirred at 0 °C for 40 min, upon which, a solution of the lactol (0.229 g, 2.20 mmol) in anhydrous THF (8 mL) was added. The reaction mixture was stirred at 0 °C for 30 min and allowed to warm to room temperature with vigorous stirring at ambient temperature for 18 h. The solution was diluted with Et<sub>2</sub>O (50 mL) and extracted with H<sub>2</sub>O (3 x 40 mL), brine (1 x 40 mL) and dried over MgSO<sub>4</sub>. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (50:50 hexanes:EtOAc) to afford 0.217 g of **2** as a colorless oil (76%).

<sup>1</sup>H NMR(300 MHz, CDCl<sub>3</sub>, 1:3 Z:E) δ 6.29 (d, J = 12.3, 1H, trans), 5.88 (dt, J = 6.3, 1.4 Hz, 1H cis), 4.72 (dt, J = 12.7, 7.3 Hz, 1H, trans), 4.33 (q, J = 7.5 Hz, 1H, cis), 3.65 (t, J = 5.8 Hz, 2H), 3.58 (s, 3H, cis), 3.50 (s, 3H, trans), 2.10 (qd, J = 7.3, 1.2 Hz, 2H, cis) 1.98 (qd, J = 7.4, 1.0 Hz, 2H, trans), 1.63-1.53 (m, 4H), 1.46-1.36 (m, 4H), 1.30 (bs, 1H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 147.250, 146.316, 106.527, 102.722, 62.893, 59.487, 55.905, 32.254, 27.433, 26.850, 25.877, 23.847. IR (cm<sup>-1</sup>): 3374, 3035, 2935, 2858, 1656.

HRMS(ESI): m/z 130.0994 (M<sup>+</sup>[C<sub>7</sub>H<sub>14</sub>O<sub>2</sub>] = 130.0992).

### (E,Z)-6-Azido-1-methoxyhexene (2)



To a solution of triphenylphosphine (0.484 g, 1.85 mmol) in anhydrous THF (14 mL) was added diethylazidodicarboxylate (0.321 g, 1.85 mmol) at 0 °C and the solution was stirred for 10 min. Alcohol **2** (0.200 g, 1.54 mmol) was added as a solution in THF (10 mL). The reaction mixture was allowed to warm to room temperature and stirred for 10 min. Diphenylphosphoryl azide (0.550 g, 2.00 mmol) was added, and the resulting solution was stirred for 24 h. The reaction was quenched with H<sub>2</sub>O (0.5 mL) and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (80:20 hexanes:EtOAc) to give 0.203 g (85%) of **3** as a colorless oil.

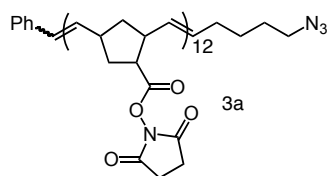
<sup>1</sup>H NMR(300 MHz, CDCl<sub>3</sub>, 1:2 Z:E) δ 6.25 (dt, J = 12.8, 1.1 Hz, 1H, trans), 5.90 (dt, J = 6.3, 1.2 Hz, 1H cis), 4.75 (dt, J = 12.8, 7.3 Hz, 1H, trans), 4.25 (q, J = 7.5 Hz, 1H, cis), 3.58 (s, 3H, cis), 3.50 (s, 3H, trans), 3.25 (t, J = 6.9 Hz, 2H), 2.10 (qd, J = 7.4, 1.4 Hz, 2H, cis) 1.98 (qd, J = 7.3, 1.3 Hz, 2H, trans), 1.67-1.56 (m, 4H), 1.48-1.37 (m, 4H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 147.49,

146.68, 105.88, 102.26, 55.932, 51.371, 28.28, 28.15, 27.79, 27.22, 26.71, 23.20 IR (cm<sup>-1</sup>): 2936, 2858, 2096, 1656.

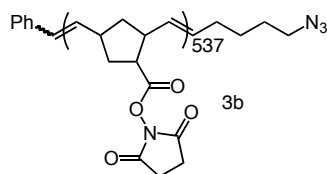
HRMS(ESI): m/z 155.1059 (M<sup>+</sup>[C<sub>7</sub>H<sub>13</sub>N<sub>3</sub>O] = 155.1063).

### General Procedure for Azide-Terminated Succinimidyl Ester Substituted Polymers 3a and 3b.

The ruthenium carbene initiator (H<sub>2</sub>IMes)(3-Br-py)<sub>2</sub>Cl<sub>2</sub>Ru=CHPh (1.13 mg, 1.26 μmol) was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (300 μL) and the mixture was added quickly to a stirring a solution of exo-bicyclo[2.2.1]hept-5-ene-2-carboxylic acid methyl ester monomer<sup>2</sup> (30.0 mg, 127 μmol) dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL) at -78 °C. The reaction was allowed to warm to room temperature and stirred for 1 h, upon which TLC indicated disappearance of the starting material. Capping agent **2** (3.0 mg, 19 μmol) was then added, and the reaction stirred at ambient temperature for 12 h. The reaction mixture was triturated using Et<sub>2</sub>O (2 x 25 ml) and the resulting residue was dried using high vacuum to yield the polymer as an off-white solid.



<sup>1</sup>H NMR(500 MHz, d<sub>6</sub>-DMSO) δ 7.39-7.09 (m, 5H, phenyl), 6.45-6.31 (m, 2H, olefin), 5.55-5.23 (m, 23H, alkene), 3.21 (bs), 2.95 (bs), 2.80 (bs), 2.64 (bs), 2.06-1.85 (m), 1.24-1.08 (m). IR (cm<sup>-1</sup>): 2946, 2098, 1780, 1737. PDI 1.20; Calculated MW 2554; M<sub>w</sub> 3084; M<sub>n</sub> 2560. DP= 12.

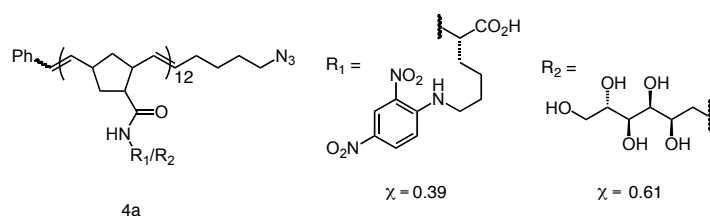


<sup>1</sup>H NMR(500 MHz, d<sub>6</sub>-DMSO) δ 7.39-7.20 (m, phenyl), 5.55-5.30 (m, alkene), 3.21 (bs), 2.95 (bs), 2.80 (bs), 2.64 (bs), 2.08-1.85 (m), 1.28-1.17 (m) IR (cm<sup>-1</sup>): 2945, 2098, 1779, 1737. PDI 1.05; Calculated MW 117871; M<sub>w</sub> 98226; M<sub>n</sub> 93320. DP = 419 .

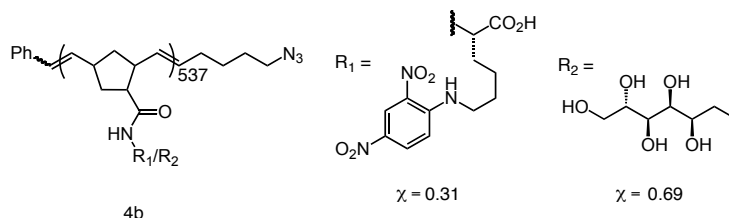
### General Procedure for DNP-Substituted Polymers 4a and 4b.

Polymer **3a** (8.40 mg, 3.30 μmol) was dissolved in anhydrous DMSO (0.500 mL), and 2,4-dinitrophenyllysine•HCl (4.59 mg, 13.0 μmol) was added and then N-methylmorpholine (3.34 mg, 37.5 μmol). This reaction mixture was stirred at room temperature for 24 h. Glucamine (11.9 mg, 65.0 μmol) dissolved in DMSO (0.200 mL) was added followed by N-methylmorpholine (3.34 mg, 37.5 μmol), and the resulting mixture was stirred for 24 h. The polymer products were purified using a PD-10 (Sephadex G-25 resin) size exclusion column using water as the eluent. The water was removed by lyophilization to afford the polymers as a yellow solid.

<sup>2</sup> Strong, L.E.; Kiessling, L.L. *J. Am. Chem. Soc.* **1999**, 121, 6193-6196.

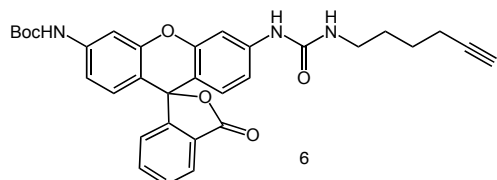


$^1\text{H NMR}$ (500 MHz,  $d_6$ -DMSO)  $\delta$  8.81 (bs, 0.39), 8.20 (bs, 0.39), 7.39-7.09 (m, 5H, phenyl), 6.45-6.31 (m, 2H, olefin), 5.55-5.230 (m, 22H, alkene) 3.32 (bs), 3.21 (bs), 2.95 (bs), 2.80 (bs), 2.64 (bs), 2.06-1.85 (m), 1.24-1.08 (m) PDI 1.19; Calculated MW 3739;  $M_w$  3890;  $M_n$  3269.



$^1\text{H NMR}$ (500 MHz,  $d_6$ -DMSO)  $\delta$  8.81 (bs, 0.32H), 8.21 (bs, 0.31H), 7.39-7.09 (m, phenyl), 7.18 (bs, 0.30H), 6.45-6.31 (m, 2H, olefin), 5.55-5.230 (m, alkene) 3.32 (bs), 3.21 (bs), 2.95 (bs), 2.80 (bs), 2.64 (bs), 2.06-1.85 (m), 1.24-1.08 (m) PDI 1.09; Calculated MW 177089;  $M_w$  153400;  $M_n$  140734.

### 5-Hexynylurea-tBoc-rhodamine<sub>110</sub> (**6**)

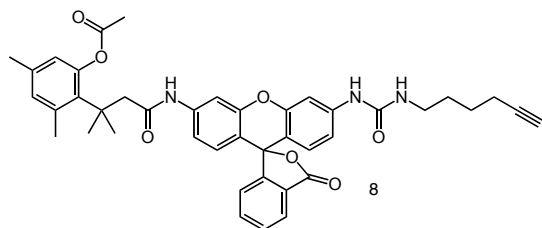


6-Heptynoic acid (47.3 mg, 0.375 mmol) was dissolved in anhydrous THF (1.0 mL) under Ar(g). The base *N,N*-diisopropylethylamine (87.0  $\mu\text{L}$ , 0.500 mmol) was added followed by diphenylphosphoryl azide (103 mg, 0.375 mmol). The resulting solution was stirred at ambient temperature for 2 h, and the reaction mixture was then heated at reflux for 3 h. The Boc-protected rhodamine **5**<sup>3</sup> (54.0 mg, 0.125 mmol) was dissolved in anhydrous THF (1.0 mL) and added to the reaction mixture. The reaction mixture was heated at reflux for 24 h. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (0-50% v/v gradient EtOAc:hexanes) to give **6** as a pale yellow crystalline solid (37.4 mg, 54%).  $^1\text{H NMR}$ (300 MHz,  $d_6$ -DMSO)  $\delta$  9.66 (s, 1H), 8.79 (s, 1H), 7.99 (d,  $J$  = 7.1 Hz, 1H), 7.82 (d,  $J$  = 2.0 Hz, 1H), 7.77 (td,  $J$  = 7.2, 1.0 Hz, 1H), 7.68 (td,  $J$  = 7.4, 1.0 Hz, 1H), 7.56 (d,  $J$  = 2.0 Hz, 1H), 7.24 (d,  $J$  = 7.4 Hz, 1H), 7.15 (dd,  $J$  = 8.6, 2.1 Hz, 1H), 7.12 (dd,  $J$  = 8.5, 2.3 Hz, 1H), 6.68 (d,  $J$  = 8.5 Hz 1H), 6.65 (d,  $J$  = 8.9 Hz, 1H), 3.08 (q,  $J$  = 6.1 Hz, 2H), 2.75 (t,  $J$  = 2.6 Hz, 1H),

<sup>3</sup> Lavis, L.D.; Chao, T-Y.; Raines, R.T. *ACS. Chem. Biol.* **2006**, 1, 252-260.

2.17 (td,  $J = 6.6, 2.6$  Hz, 2H), 1.70-1.63 (m, 2H), 1.52-1.43 (m, 2H), 1.49 (s, 9H).  $^{13}\text{C}$  NMR (75 MHz,  $d_6$ -DMSO)  $\delta$  169.39, 155.61, 153.29, 153.21, 151.86, 151.82, 143.61, 142.56, 136.25, 130.75, 128.93, 128.82, 126.64, 125.35, 124.61, 114.99, 114.69, 112.80, 111.52, 105.71, 104.96, 85.04, 83.18, 80.31, 71.82, 39.17, 29.54, 28.69 (3C), 26.06, 18.13.  
HRMS (ESI):  $m/z$  576.2111 ( $\text{MNa}^+ [\text{C}_{32}\text{H}_{31}\text{N}_3\text{O}_6\text{Na}] = 576.2116$ ).

### 5-Hexynylurea-rhodamine<sub>110</sub> trimethyl lock (8)



The urea-substituted rhodamine derivative (25.0 mg, 0.055 mmol) was dissolved in a 1:1 DMF:pyridine mixture (0.50 mL) under Ar(g). A solution of N-(3-dimethylamino-propyl)-N'-ethylcarbodiimide hydrochloride (21.1 mg, 0.110 mmol) and trimethyl lock derivative **7**<sup>4</sup> in 1:1 DMF:pyridine (0.50 mL) was added. The resulting reaction mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure, and the pale orange residue purified by flash chromatography (0-50% gradient EtOAc:hexanes) to afford a white crystalline solid (27.3 mg, 71%).

$^1\text{H}$  NMR(300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.97 (d,  $J = 6.7$  Hz, 1H), 7.82 (bs, 1H), 7.62 (td,  $J = 7.4, 1.4$  Hz, 1H), 7.57 (td,  $J = 7.4, 1.1$  Hz, 1H), 7.39 (bs, 2H), 7.05 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.03 (m, 1H), 6.88 (d,  $J = 2.0$  Hz, 1H) 6.79 (d,  $J = 1.7$  Hz, 1H), 6.68 (dd,  $J = 8.7, 2.0$  Hz, 1H), 6.62 (d,  $J = 1.5$  Hz, 1H), 6.56 (d,  $J = 8.7$  Hz, 1H), 6.49 (d,  $J = 8.8$  Hz, 1H), 5.64 (t,  $J = 5.6$  Hz, 1H), 3.21 (q,  $J = 6.2$  Hz, 2H), 2.64 (ABq,  $J = 14.3$  Hz, 2H), 2.47 (s, 3H), 2.37 (s, 3H), 2.21 (s, 3H), 2.18-2.2 (m, 2H), 1.91(t,  $J = 2.6$  Hz, 1H), 1.69 (s, 3H), 1.68 (s, 3H), 1.67-1.50 (m, 4H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.97, 170.37, 170.34, 155.30, 153.21, 151.51, 151.44, 150.04, 141.87, 139.98, 138.88, 137.26, 135.35, 133.16, 132.95, 129.81, 128.30, 127.99, 126.17, 124.93, 124.15, 123.47, 115.34, 115.04, 113.69, 111.53, 107.71, 106.27, 84.16, 83.95, 77.22, 68.63, 50.99, 40.32, 39.51, 32.10, 29.20, 25.72, 25.57, 21.95, 20.18, 18.14

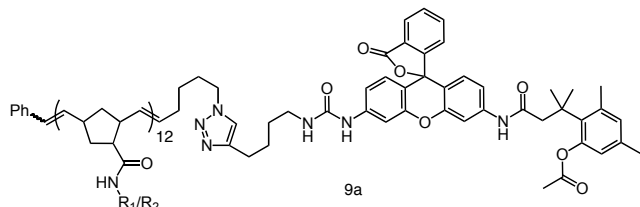
HRMS (ESI):  $m/z$  700.3023 ( $\text{MH}^+ [\text{C}_{42}\text{H}_{41}\text{N}_3\text{O}_7] = 700.3030$ ).

### General Procedure for Cu-Catalyzed Azide-Alkyne Cycloaddition Reaction to Form Pro-fluorophore Conjugated Polymers **9a** and **9b**

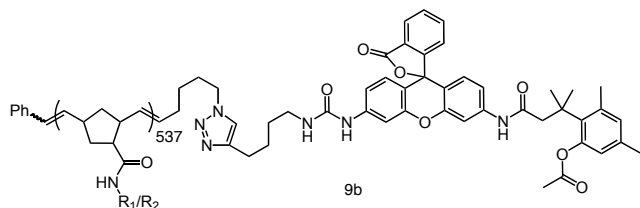
The DNP-substituted polymer **4a** (1.60 mg, 0.457  $\mu\text{mol}$ ) and the trimethyl lock-masked pro-fluorophore **8** (5.2 mg, 7.5  $\mu\text{mol}$ ) were dissolved in a mixture of 2:1 DMSO:H<sub>2</sub>O (50  $\mu\text{l}$ ). To this was added  $\text{CuSO}_4 \cdot \text{pentahydrate}$  (11  $\mu\text{g}$ , 0.045  $\mu\text{mol}$ ) and sodium ascorbate (18  $\mu\text{g}$ , 0.090  $\mu\text{mol}$ ). The reaction mixture was stirred at room temperature for 20 min and tris(benzyltriazolymethyl)amine (24.0  $\mu\text{g}$ , 0.045  $\mu\text{mol}$ ) was added. The solution was purged with nitrogen and stirred at ambient temperature for 24 h. Polymers were purified by a PD-10

<sup>4</sup> Amsberry, K.L.; Gerstenberger, A.E.; Borchardt, R.T. *Pharm. Res.* **1991**, *8*, 455-461.

(Sephadex G-25 resin) size exclusion column using water as the eluent. Extensive dialysis (MW cutoff 1000) against deionized water was used for further purification of the 10-mer.



$^1\text{H NMR}$ (500 MHz,  $d_6$ -DMSO)  $\delta$  8.85 (bs) 8.78 (s), 8.24 (bs), 8.00 (d,  $J = 7.6$  Hz, 1H), 7.80-7.70 (m), 7.59 (bs), 7.36-7.21 (m), 7.08 (d,  $J = 8.7$  Hz, 1H), 6.94 (d,  $J = 8.3$  Hz, 1H), 6.80 (s, 1H), 6.67 (d,  $J = 8.6$  Hz, 1H), 6.59 (bs, 1H), 6.35 (m), 6.24 (bs), 5.32-5.16 (m, olefin), 4.78-4.70 (m), 4.45-4.16 (m), 3.56 (bs), 2.94 (bs), 2.37 (bs), 2.27 (bs), 2.16 (bs), 1.0-1.84 (m), 1.60-1.36 (m), 1.05 (bs).

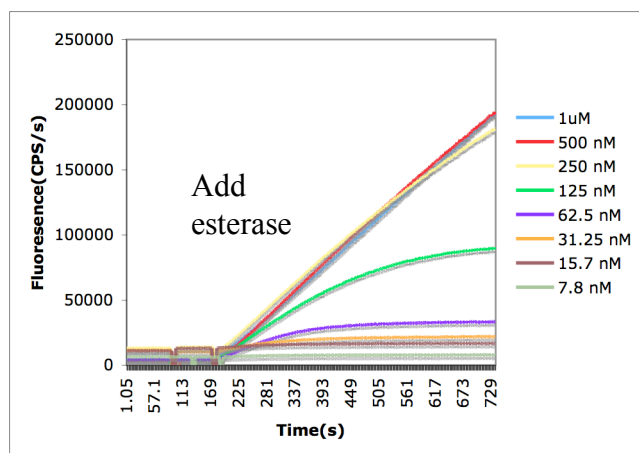


$^1\text{H NMR}$ (500 MHz,  $d_6$ -DMSO)  $\delta$  8.79 (bs, H), 8.20 (bs, H), 7.98 (d,  $J = 7.2$  Hz, H), 7.76 (t,  $J = 7.3$  Hz, 1H), 7.71 (t,  $J = 7.3$  Hz, 1H), 7.69-7.61 (m, H), 7.57 (bs, H), 7.19 (d,  $J = 7.4$  Hz, 1H), 7.15 (bs, H), 7.03 (d,  $J = 8.03$  Hz, 1H), 6.91 (dd,  $J = 8.6, 1.9$  Hz, 1H), 6.78 (s, H), 6.63 (d,  $J = 8.5$  Hz, 1H), 6.57 (d,  $J = 8.8$  Hz, 1H), 6.55 (bs, H), 6.24 (bs, H), 5.28-5.15 (m, olefin), 4.78-4.70 (m, H), 4.45-4.16 (m), 3.56 (bs), 2.94 (bs), 2.37 (bs), 2.27 (bs), 2.16 (bs), 1.0-1.84 (m), 1.60-1.36 (m), 1.05 (bs).

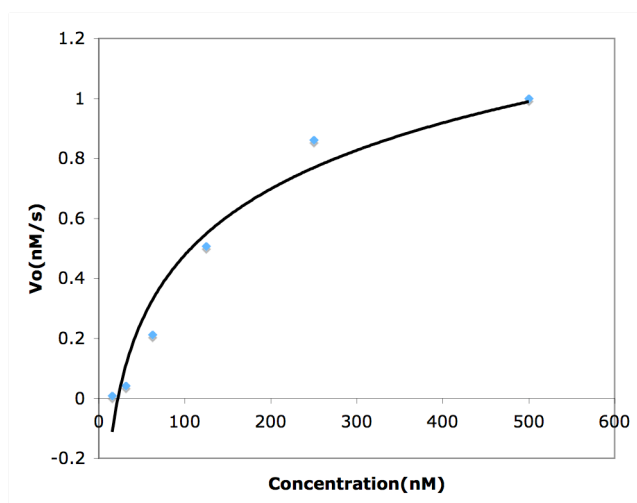
**Fluorescence Microscopy.** B cells that display the DNP-specific B cell receptor (A20.2J/HL<sub>TNP</sub> cells) were added to a chambered coverslip at  $1 \times 10^5$  cells/well and allowed to adhere for 1 h. The cells were stained with 13  $\mu\text{g}/\text{mL}$  Cy3-conjugated anti-IgM (Jackson Immunoresearch) for 30 min on ice to label the DNP-specific B cell receptor. Stained cells were washed (3x) with phosphate-buffered saline containing 1% bovine serum albumin and 1 mM  $\text{CaCl}_2$ . Samples were warmed to 37  $^\circ\text{C}$  using a heated stage. The buffer was removed and replaced with pre-warmed buffer containing **9a** or **9b**. The concentrations used (5  $\mu\text{M}$  DNP) were based on DNP-epitopes and determined by UV absorbance. Images were collected immediately after addition of polymer and after 15 minutes. The results shown were not isolated events; images shown are representative of observations in multiple fields of view. Images were collected using an inverted Nikon Eclipse TE2000 confocal microscope with a 60x (1.4 NA) oil immersion lens. To reduce background noise, Kalman mode ( $n=3$ ) was used with a scan speed of 166 lps. **A-B** zoom: 1x, **C-F** zoom: 4x. The pinhole was set to 2.6 for green and 2.5 for red resulting in a slice thickness of 1.02  $\mu\text{m}$  for both fluorescent channels. Images were processed using Adobe Photoshop CS2 (brightness adjusted using autolevels, color converted to RGB, overlaid).

**Kinetics of Fluorophore Unmasking:** All kinetic evaluations were performed in phosphate-buffered saline (PBS, pH 7.3), which contained (in 1 L) KCl (0.2 g),  $\text{KH}_2\text{PO}_4$  (0.2 g), NaCl (8.0 g), and  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$  (2.16 g). Pig liver esterase (PLE; MW 163 kDa) was obtained from Sigma Chemical (St. Louis, MO; product number E2884) as a suspension in 3.2 M ammonium sulfate buffer, and was diluted to appropriate concentrations in PBS before use. Stock solutions of pro-fluorophore **9a** were prepared in DMSO and added to PBS for the kinetic experiments such that DMSO concentrations never exceeded 1% (v/v). Fluorometric measurements were made with using fluorescence grade quartz or glass cuvettes from Starna Cells (Atascadero, CA) and a QuantaMaster1 photon-counting spectrofluorometer from Photon Technology International (South Brunswick, NJ) equipped with sample stirring.

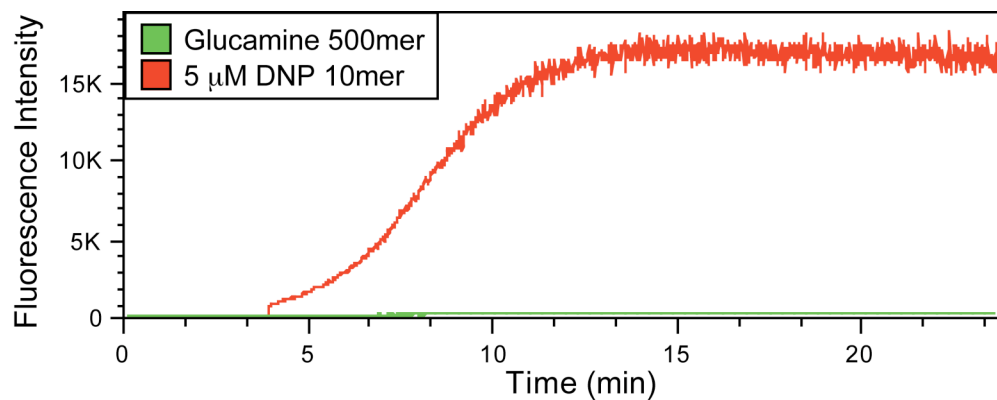
(a)



(b)



Kinetic traces (a) ( $\lambda_{\text{ex}} = 496 \text{ nm}$ ,  $\lambda_{\text{em}} = 520 \text{ nm}$ ) and Michaelis-Menten plot (b) for a serial dilution of profluorophore **9a** (1.0  $\mu\text{M}$   $\rightarrow$  7.8 nM) with PLE (5  $\mu\text{M}^{-1}$ ).



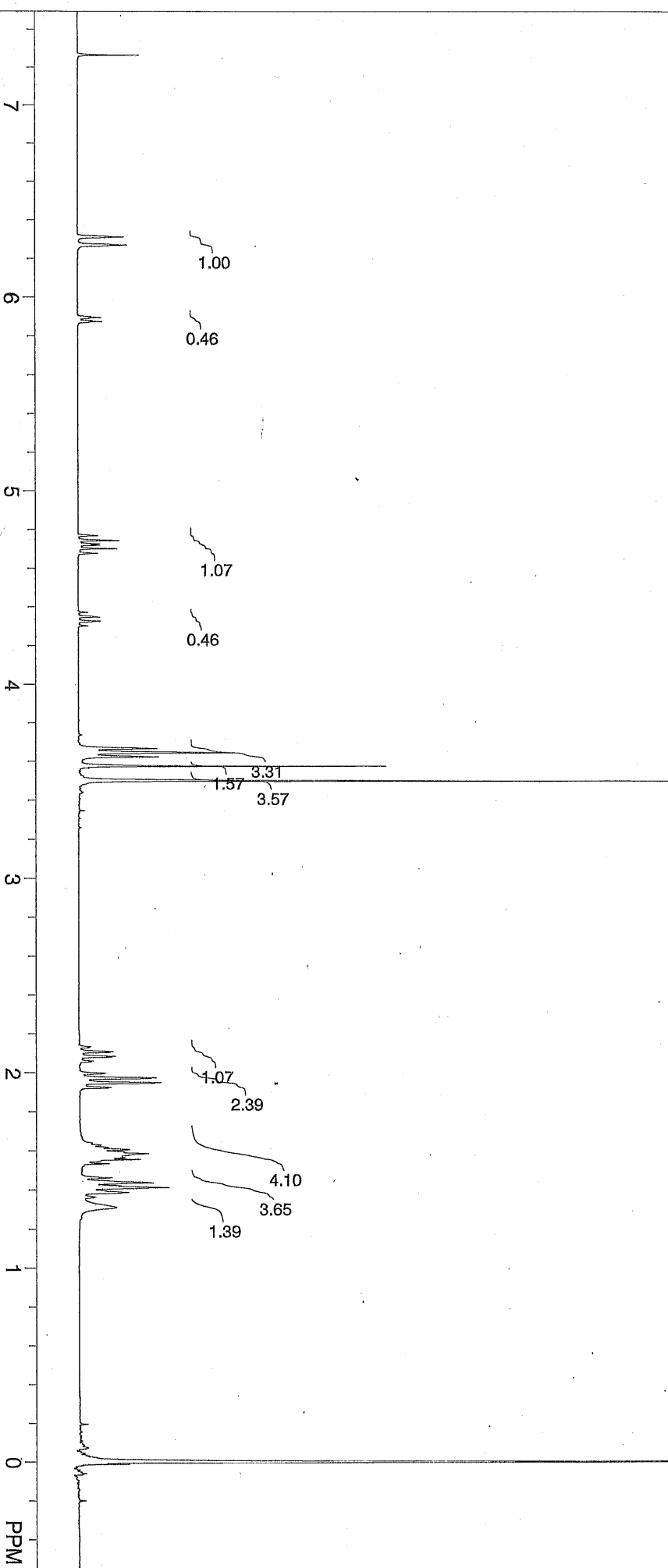
A20.2J/HLTNP cells were resuspended at  $\sim 1 \times 10^6$  cells/mL in PBS with 1% BSA and 1 mM  $\text{CaCl}_2$ . Cell associated fluorescence was measured using an LSRII flow cytometer (Becton Dickinson) with FACSDiva software. After establishing a baseline for 4 min, cells were stimulated with the indicated polymer and monitored for an additional 20 min. Cells were kept at 37 °C by a recirculating water jacket. Data were analyzed using the FlowJo software package (Tree Star, Inc.).





CDCl<sub>3</sub>

300.13 MHz

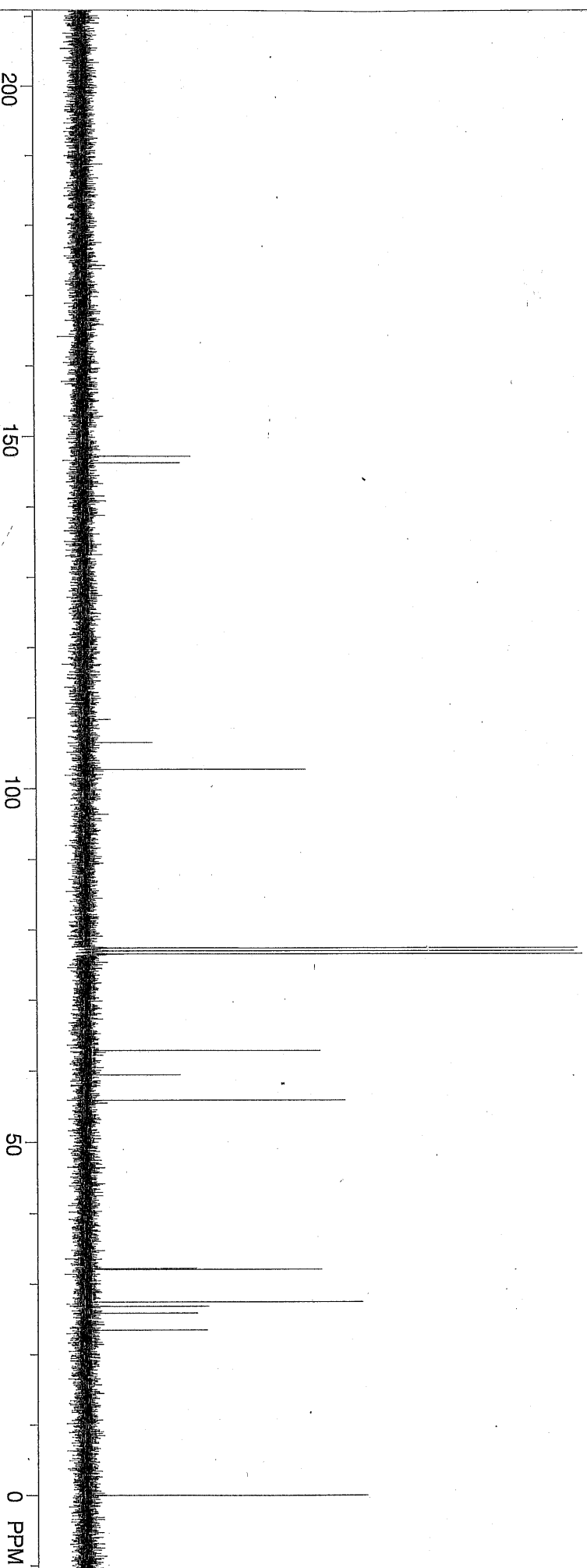


:blank line/export/home/walkup/auto\_2007.01.30/mangold\_SLM1017\_10\_01  
F1: 299.732 F2: 75.375 SW1: 5995 SW2: 5995  
EX: s2pul PW: 4.9 us PD: 0.1 sec NA: 16 IB: 0.3  
OFT: 2099.5  
PTS1d: 23981 32768  
USER: -- DATE: Jan 30 2007  
Nuts - \$Proton 01.fid



CDCl<sub>3</sub>

299.73 MHz

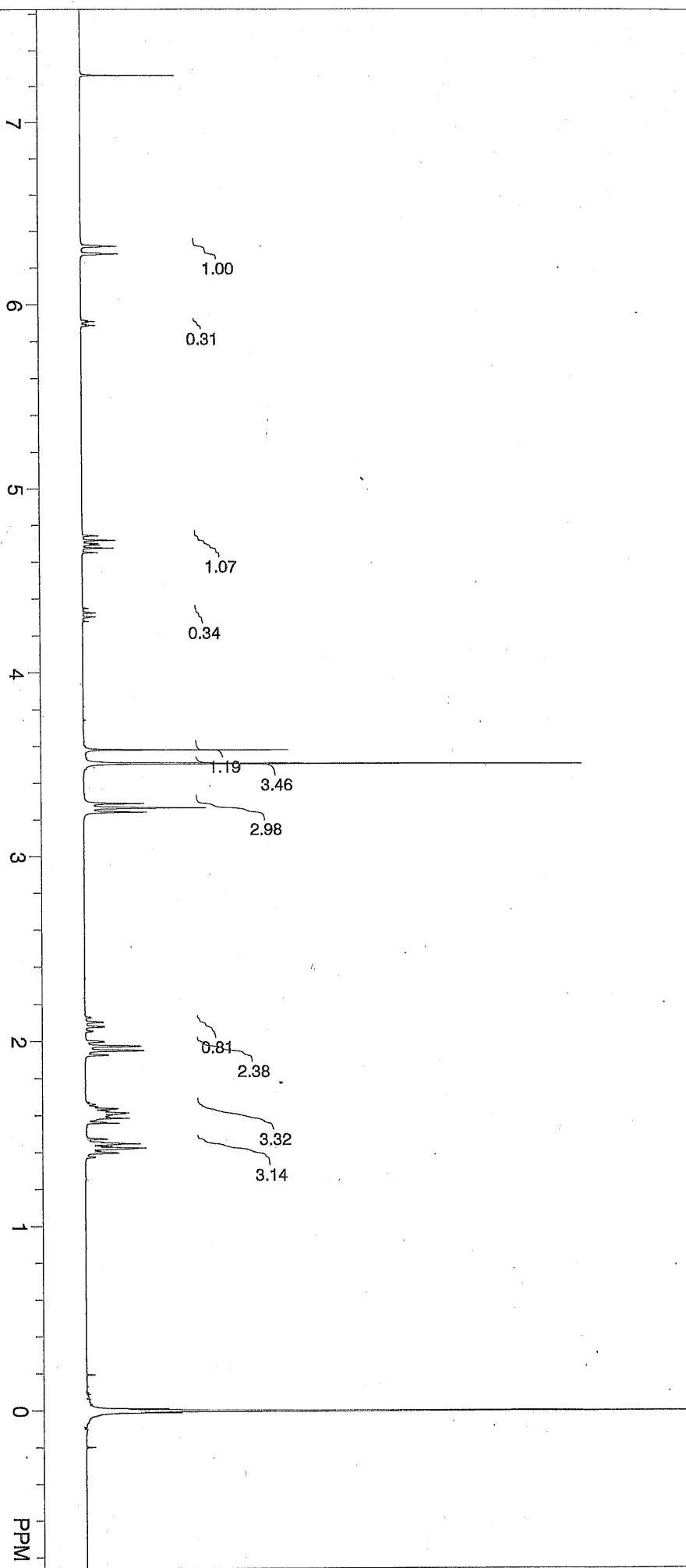


.blank line/export/home/walkup/auto\_2007.01.30/mangold\_SIMI017\_10\_01  
F1: 75.375 F2: 299.731 SW1: 19608 OF1: 8274.8  
EX: s2pul PW: 5.8 us PD: 2.0 sec NA: 256 IB: 0.0 PTS1d: 29412 , 32768  
USER: -- DATE: Jan 30 2007  
Nuts - \$Carbon 01.fid



CDCl<sub>3</sub>

300.13 MHz

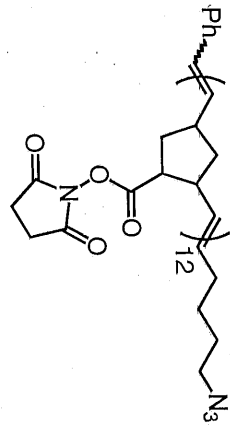


F1: 300.135	F2: 1.000	SW1: 6024	PD: 0.0 sec	OF1: 1733.2	LB: 0.3	PTS1d: 16384 , 32768
EX:		PW: 3.0 us		NA: 8		

USER: -- DATE: 08/21/1987

Nuts - \$SIM1088.006





d<sub>6</sub>-DMSO

500.23 MHz

8 6 4 2 0 PPM

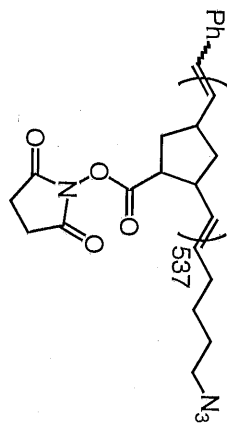
5.00  
1.32  
23.51  
166.37  
39.48  
16.19

STANDARD PROTON PARAMETERS

F1: 500.231	F2: 125.794	SW1: 8003	PD: 1.0 sec	OF1: 3001.3	IB: 0.3	PTS1d: 31760 , 32768
EX: s2pul		PW: 4.8 us		NA: 2052		

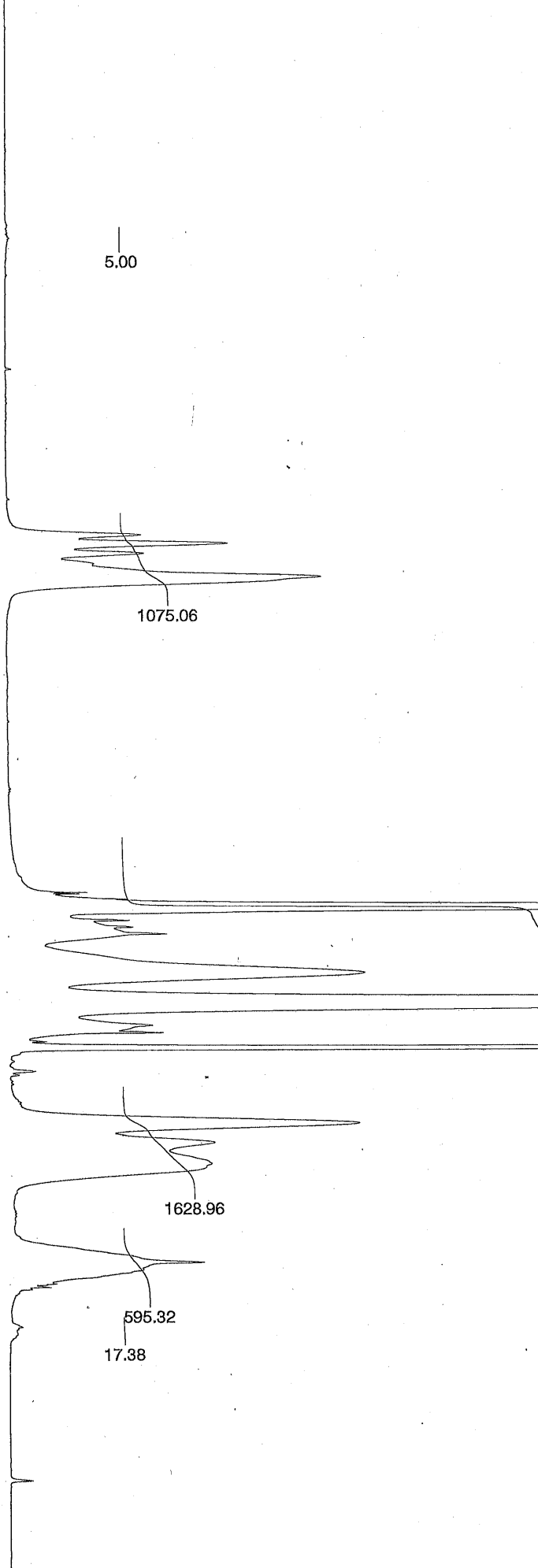
USER: -- DATE: Aug 24 2007

Nuts - \$10mer azide.fid



d<sub>6</sub>-DMSO  
500.23 MHz

8  
7  
6  
5  
4  
3  
2  
1  
0 PPM

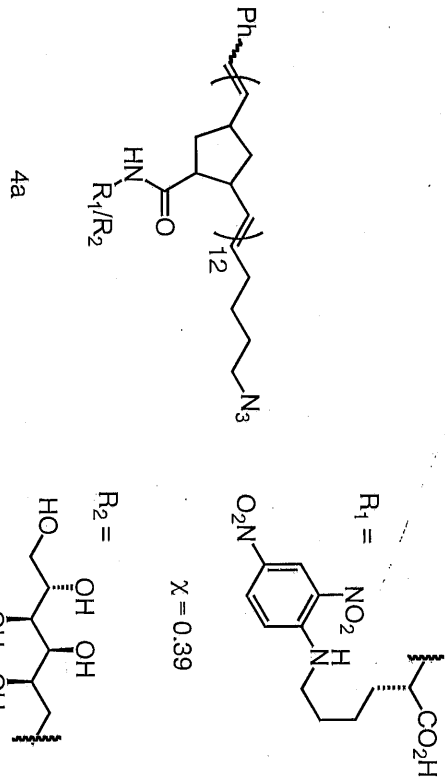


STANDARD PROTON PARAMETERS

F1: 500.231	F2: 125.794	SW1: 8003	PD: 1.0 sec	OP1: 3001.3	PTS1d: 31760 , 32768
EX: s2pul		PW: 4.8 us		NA: 11692	IB: 0.3

USER: -- DATE: Sep 2 2007

Nuts - \$500mer Azide.fid



d<sub>6</sub>-DMSO  
500.23 MHz

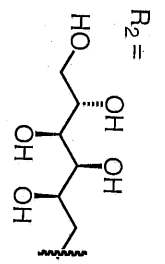
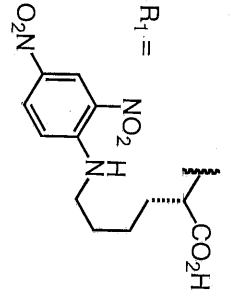
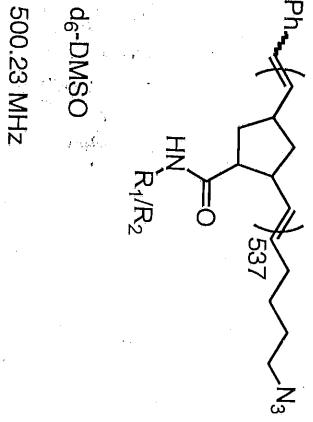
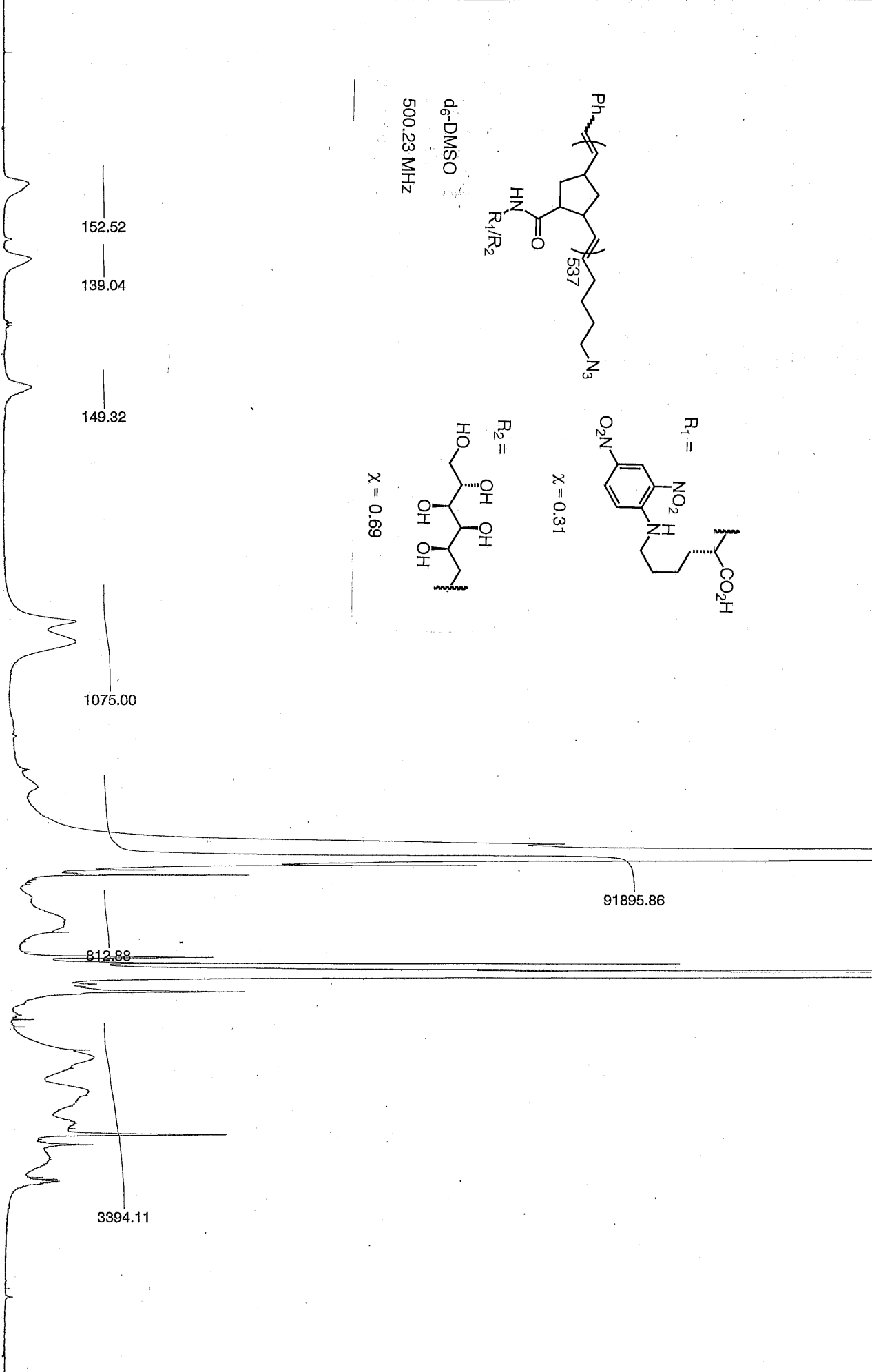


STANDARD PROTON PARAMETERS

F1: 500.231	F2: 125.794	SW1: 8003	PD: 1.0 sec	OF1: 3001.3	IB: 0.3	PTS1d: 31760	32768
EX: s2pul		PW: 4.8 us		NA: 8204		Nuts - \$SM-I-130-3-DNP10mer.fid	

USER: -- DATE: Oct 25 2007

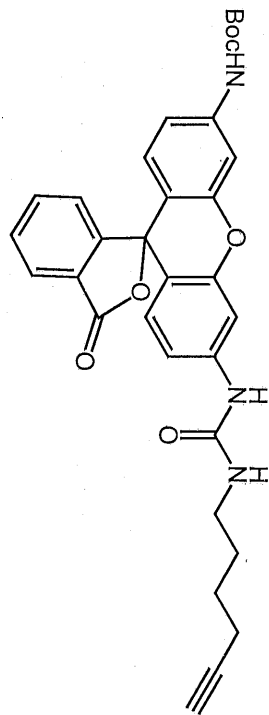
10  
8  
6  
4  
2  
0 PPM



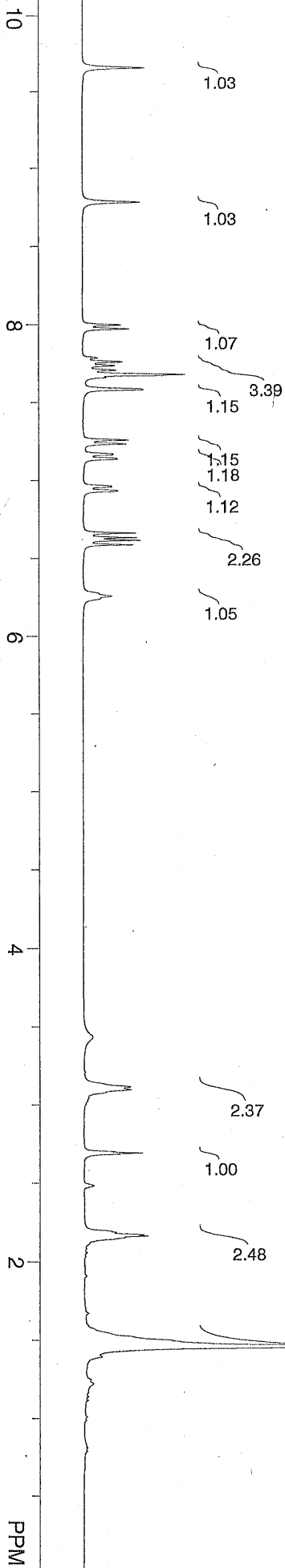
STANDARD PROTON PARAMETERS

F1: 500.231	F2: 125.794	SW1: 8003	PD: 1.0 sec	OF1: 3001.4	LB: 0.3	PTSID: 31760	USER: -- DATE: Jun 12 2007
EX: s2pul		PW: 4.8 us		NA:		32768	Nuts - \$SM-I-59-500mer-DNP.fid





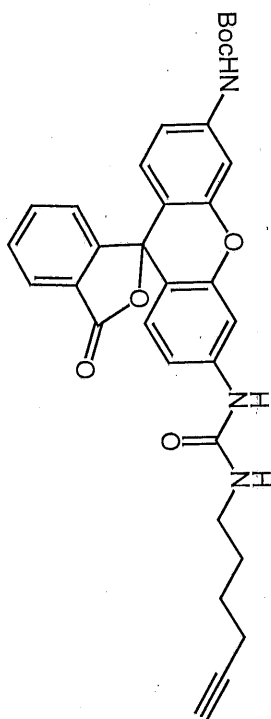
d<sub>6</sub>-DMSO  
299.73 MHz



blank line/export/home/walkup/auto\_2007.03.23/mangold\_Fluorophore\_Alkyne\_E  
 F1: 299.733 F2: 75.375 SW1: 5995 PD: 0.1 sec OF1: 2098.1 NA: 32 IB: 0.3 PTS1d: 23981 , 32768  
 EX: s2pul PW: 4.9 us

USER: -- DATE: Mar 25 2007

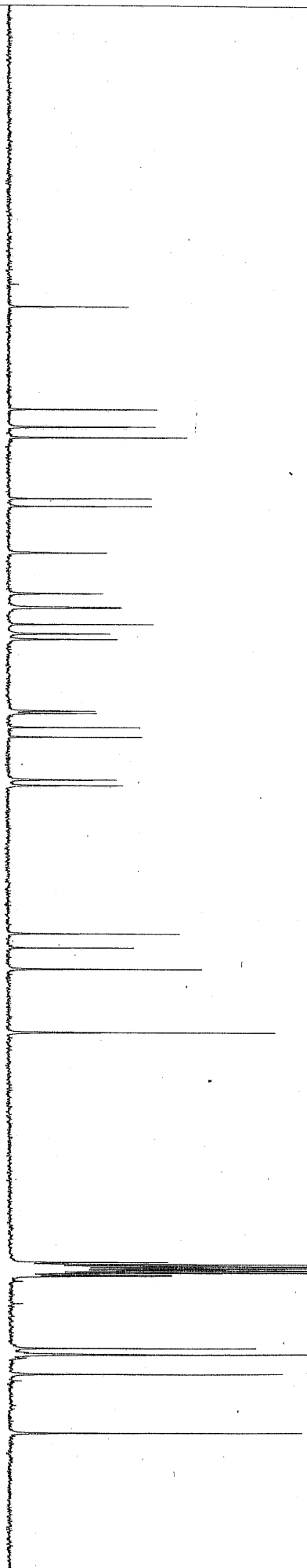
Nuts - \$Proton 01.fid



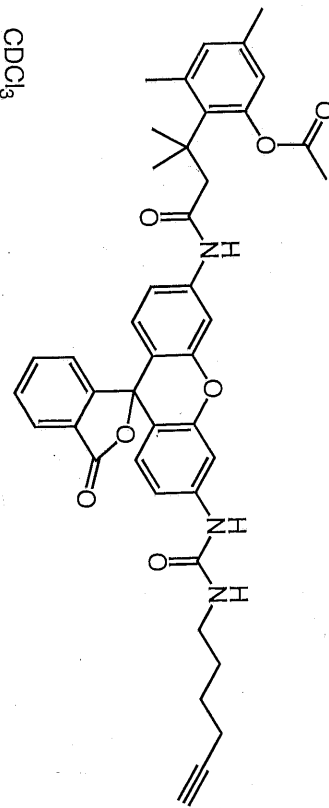
$d_6$ -DMSO

299.73 MHz

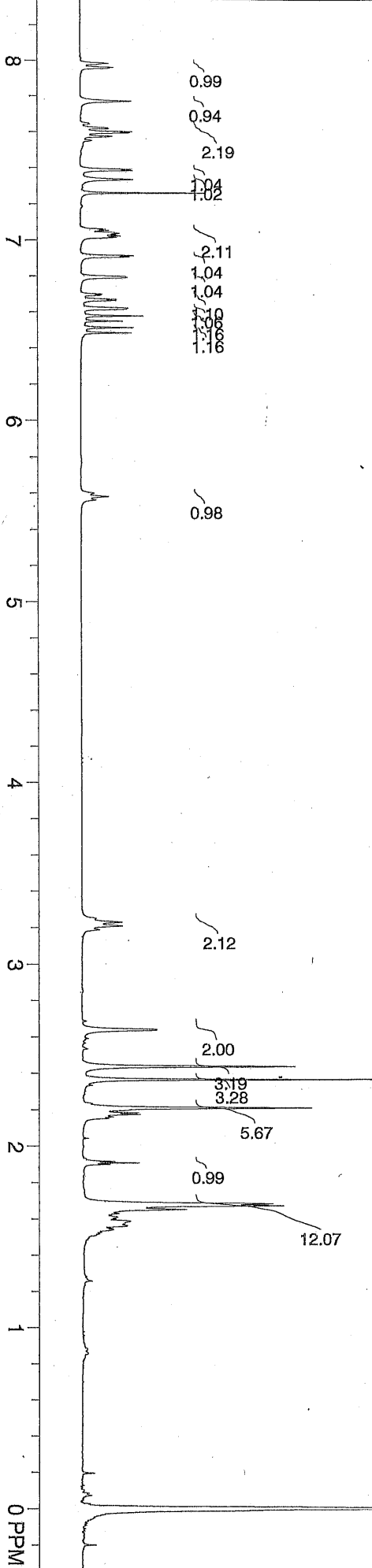
200 150 100 50 PPM



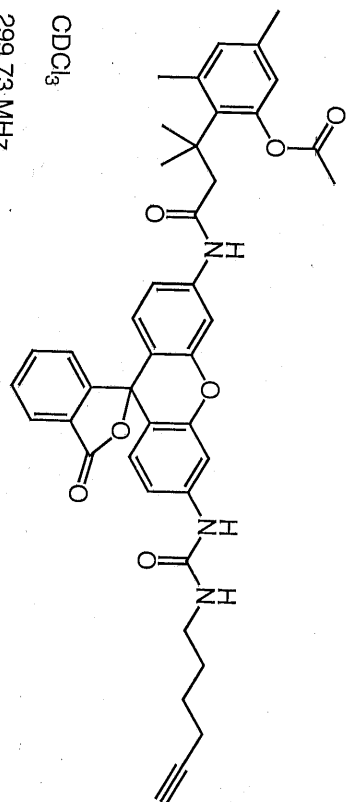
blank line/export/home/walkup/auto\_2007.03.23/mangold\_Fluorophore\_Alkyne\_E  
 F1: 75.376 F2: 299.733 SW1: 1.19608 OF1: 8290.4  
 EX: s2pul PW: 5.8 us PD: 2.0 sec NA: 5000 LB: 2.0  
 PTS1d: 29412 , 32768  
 USER: -- DATE: Mar 25 2007  
 Nuts - \$Carbon 01.fid



CDCl<sub>3</sub>  
299.73 MHz

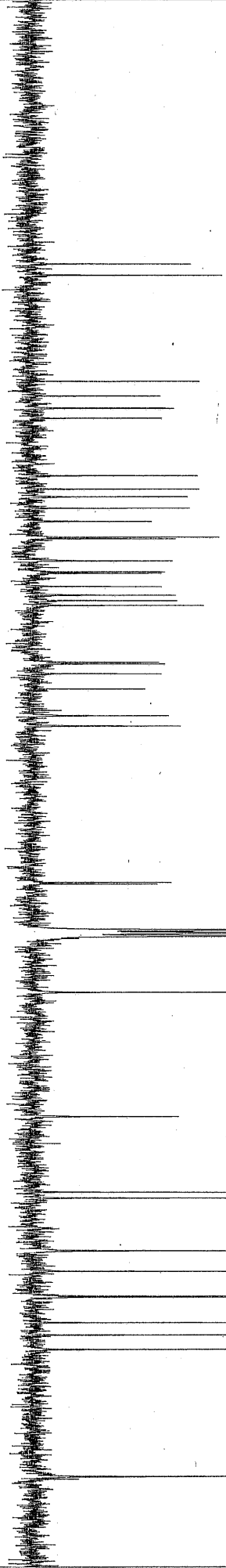


blank line/export/home/walkup/auto\_2007.06.06/mangold\_Trimethyllock\_Flurop  
 FT: 299.732 F2: 75.375 SW1: 5995 PW: 4.9 us PD: 0.1 sec NA: 32 LB: 0.3  
 USER: -- DATE: Jun 7 2007  
 PTS1d: 23981 32768  
 Nuts - \$Proton 01.fid

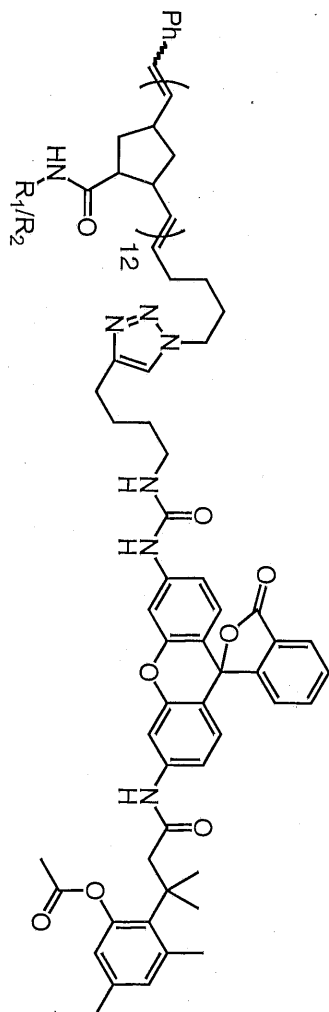


CDCl<sub>3</sub>  
299.73 MHz

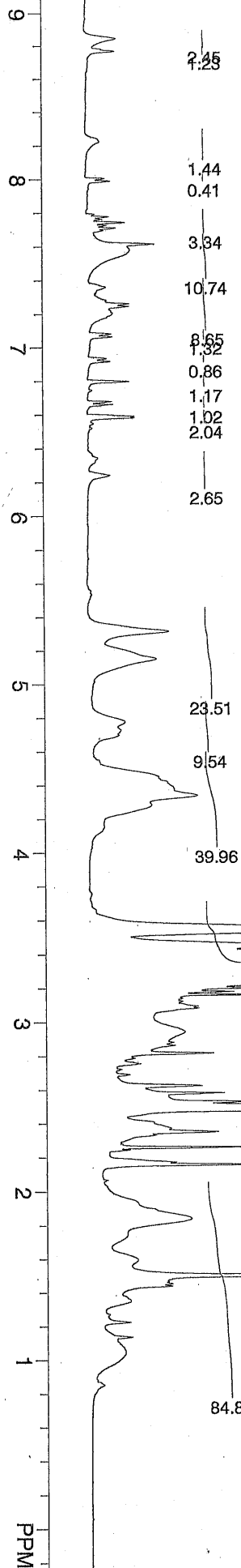
200 150 100 50 0 PPM



Blank line/export/home/walkup/auto\_2007.06.06/mangold\_Trimethyllock\_Flurop  
 F1: 75.375 F2: 299.731 SW1: 19608 OF1: 8275.1  
 EX: s2pul PW: 5.8 us PD: 2.0 sec NA: 2000 IB: 2.0  
 PTSId: 29412 , 32768  
 USER: -- DATE: Jun 7 2007  
 Nuts - \$Carbon 01.fid

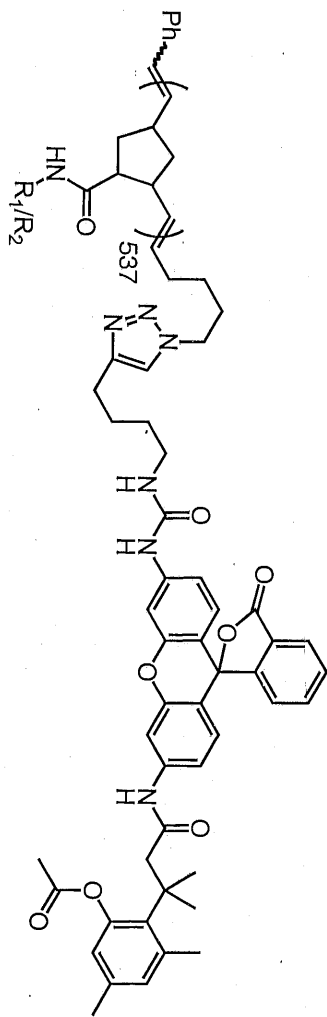


d<sub>6</sub>-DMSO  
500.23 MHz

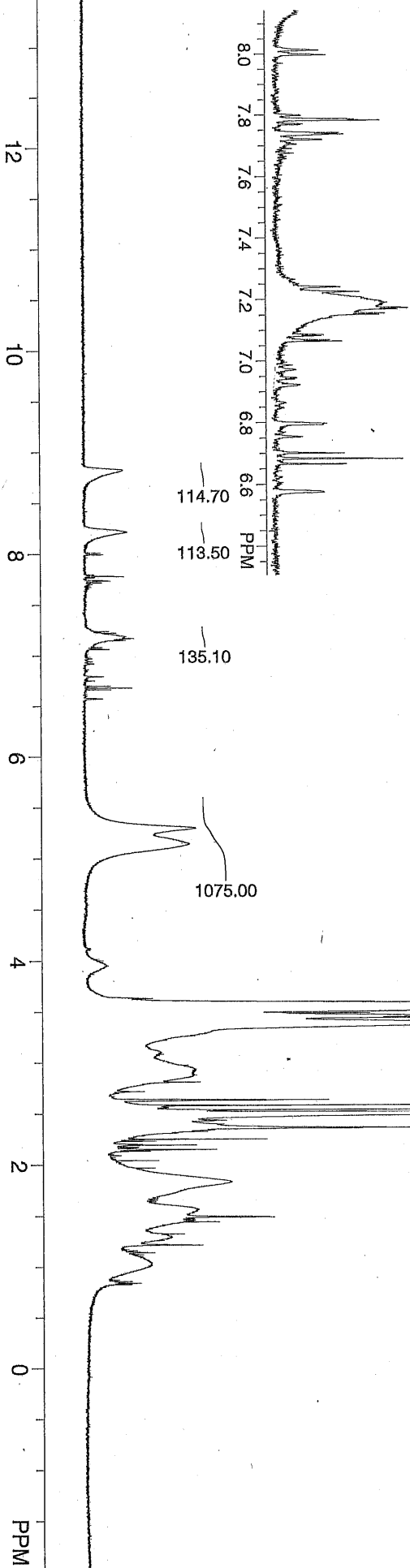


STANDARD PROTON PARAMETERS

F1: 500.231	F2: 125.794	SW1: 8003	PD: 1.0 sec	OP1: 3001.3	PTS1d: 31760
EX: s2pul		PW: 4.8 us		NA: 11360	IB: 0.3
USER: -- DATE: Aug 30 2007					
Nuts - \$Clicked 10mer.fid					



$d_6$ -DMSO  
500.23 MHz



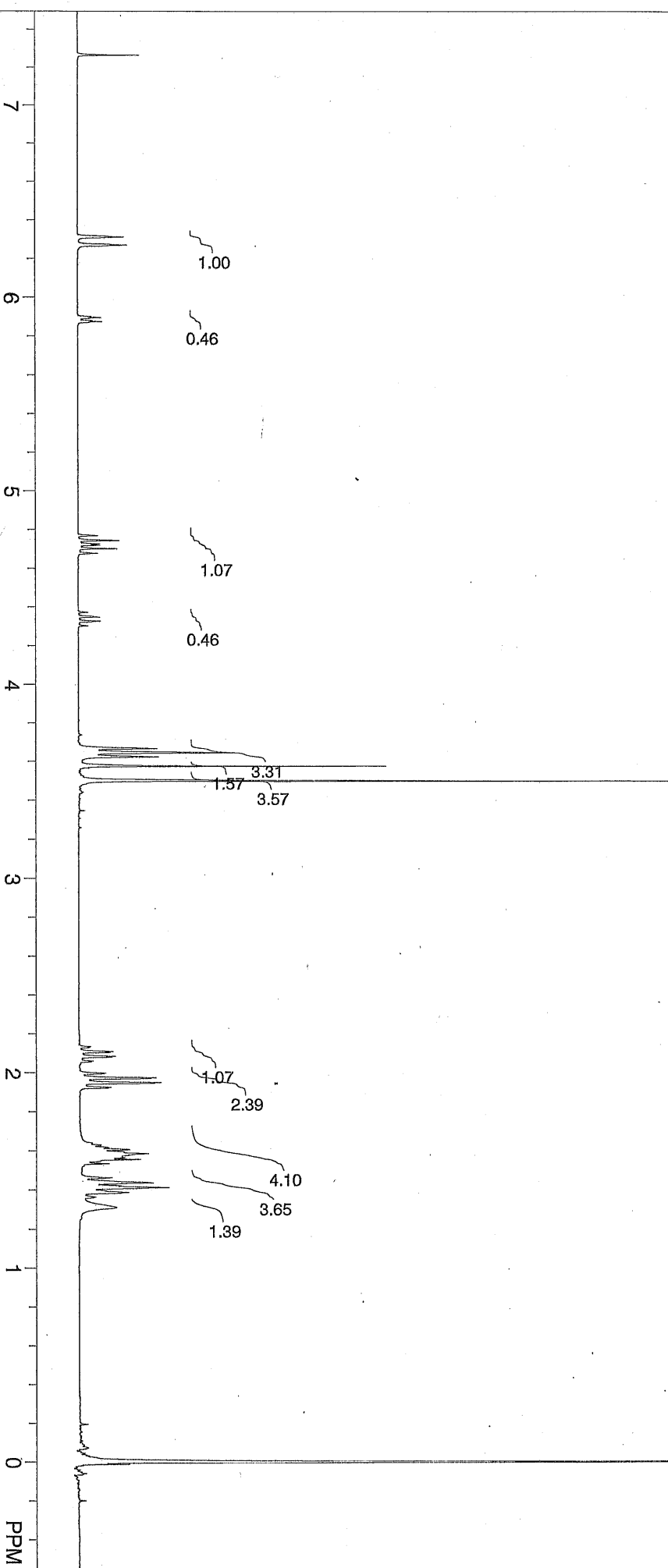
STANDARD PROTON PARAMETERS

F1: 500.231	F2: 125.794	SW1: 8003	PD: 1.0 sec	OF1: 3001.3	LB: 0.3	PTSid: 31760 , 32768	USER: -- DATE: May 22 2008
EX: s2nul		PW: 4.8 us		NA: 800			Nuis - \$clicked500mer.fid



CDCl<sub>3</sub>

300.13 MHz



:blank line/export/home/walkup/auto\_2007.01.30/mangold\_SLM1017\_10\_01  
F1: 299.732 F2: 75.375 SW1: 5995 PW: 4.9 us PD: 0.1 sec OF1: 2099.5 NA: 16 IB: 0.3 PTS1d: 23981 32768  
EX: s2pul

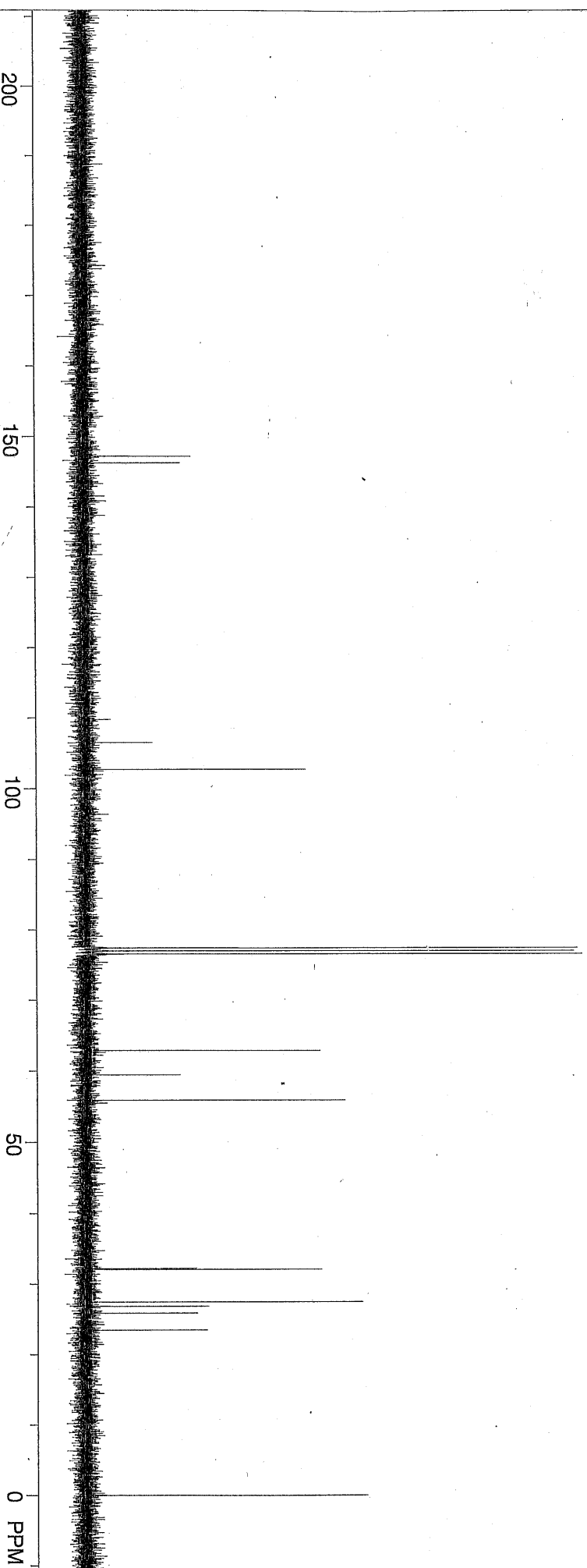
USER: -- DATE: Jan 30 2007

Nuts - \$Proton 01.fid



CDCl<sub>3</sub>

299.73 MHz



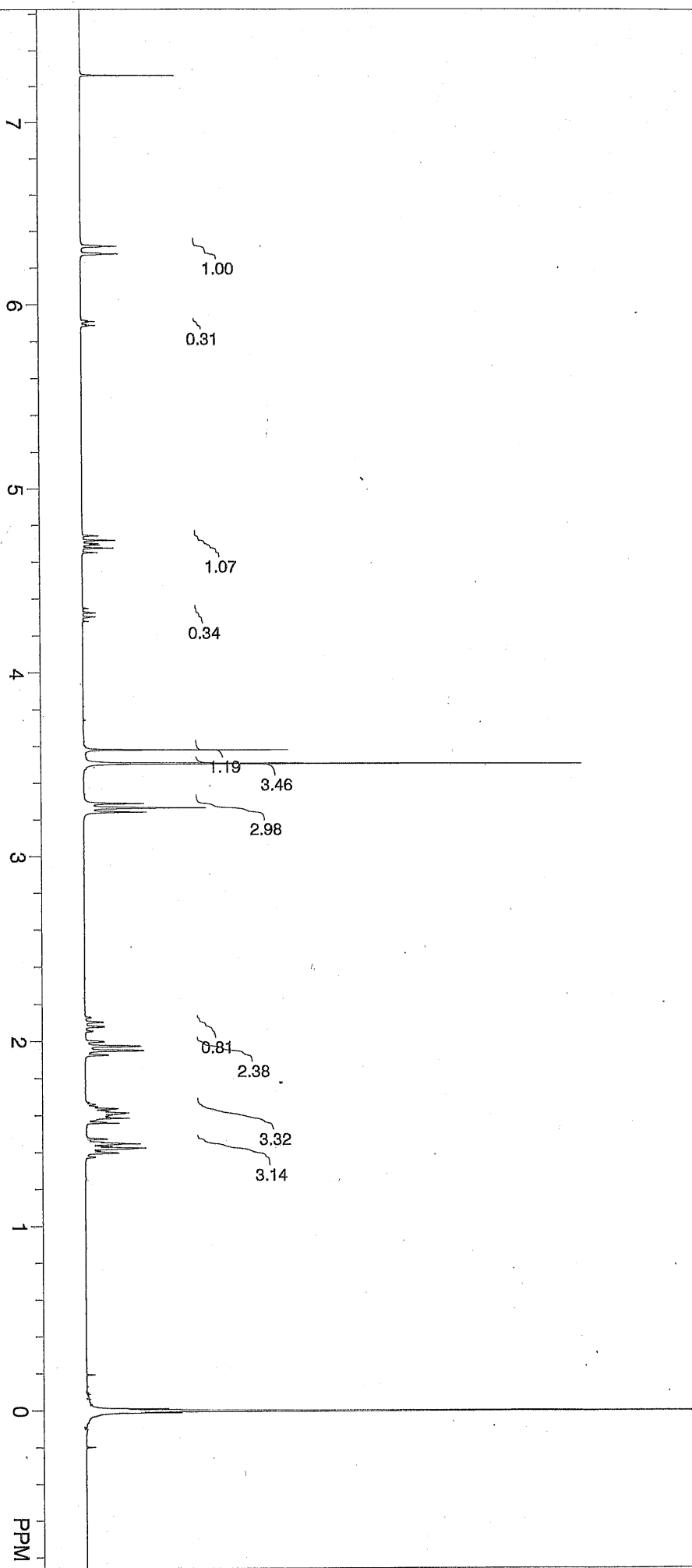
.blank line/export/home/walkup/auto\_2007.01.30/mangold\_SIMI017\_10\_01  
F1: 75.375 F2: 299.731 SW1: 19608 OF1: 8274.8  
EX: s2pul PW: 5.8 us PD: 2.0 sec NA: 256 IB: 0.0 PTS1d: 29412 , 32768  
USER: -- DATE: Jan 30 2007  
Nuts - \$Carbon 01.fid





CDCl<sub>3</sub>

300.13 MHz



F1: 300.135 F2: 1.000 SW1: 6024 PD: 0.0 sec OF1: 1733.2 NA: 8 LB: 0.3 PTS1d: 16384, 32768  
 EX: PW: 3.0 us

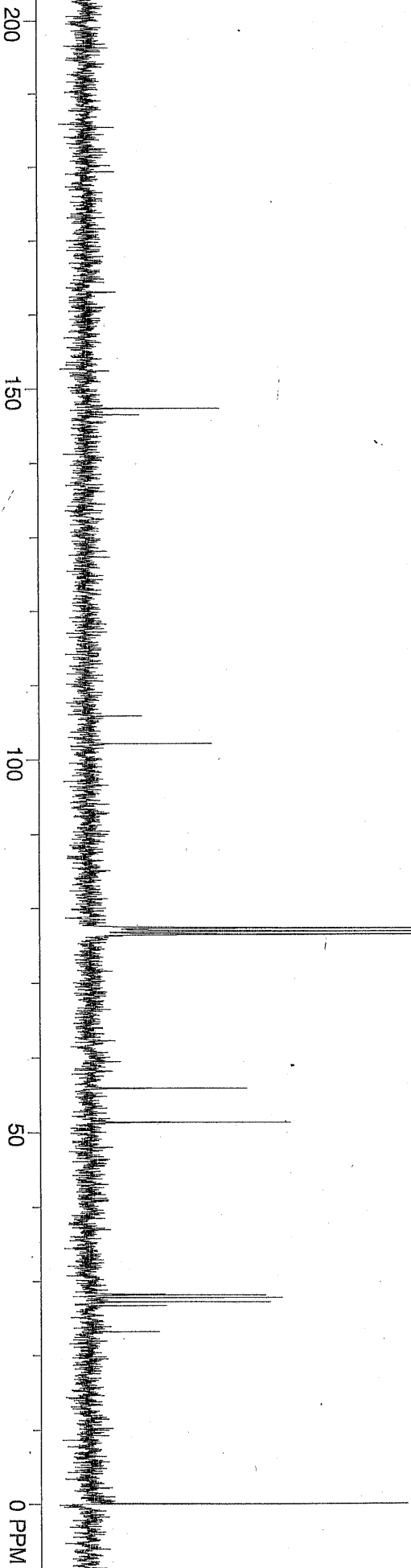
USER: -- DATE: 08/21/1987

Nuts - \$SIM1088.006

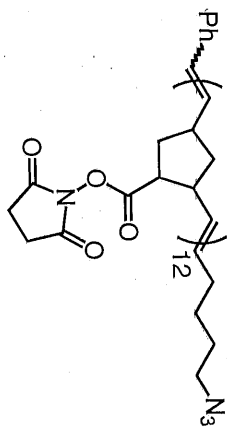


CDCl<sub>3</sub>

299.73 MHz



blank line/export/home/walkup/auto\_2007.01.30/mangold\_SLM1018\_18\_01  
F1: 75.375 F2: 299.731 SW1: 19608 OF1: 8274.5  
EX: s2pul PW: 5.8 us PD: 2.0 sec NA: 256 LB: 2.0  
PTS1d: 29412 , 32768  
USER: -- DATE: Jan 31 2007  
Nuts - \$Carbon 01.fid



d<sub>6</sub>-DMSO

500.23 MHz

8 6 4 2 0 PPM

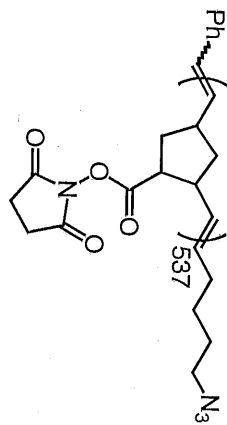
5.00  
1.32  
23.51  
166.37  
39.48  
16.19

STANDARD PROTON PARAMETERS

F1: 500.231	F2: 125.794	SW1: 8003	PD: 1.0 sec	OF1: 3001.3	IB: 0.3	PTS1d: 31760 , 32768
EX: s2pul		PW: 4.8 us		NA: 2052		

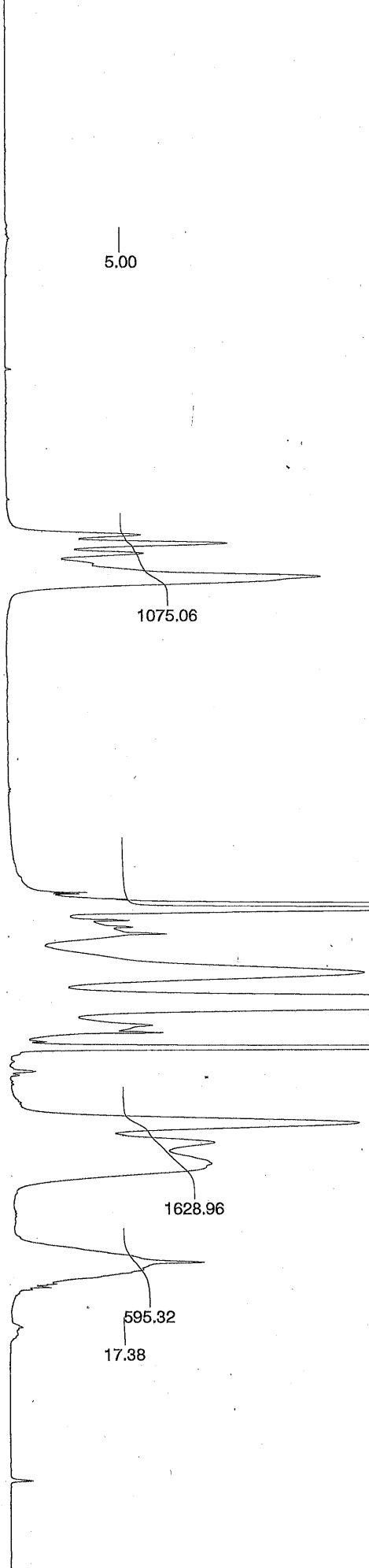
USER: -- DATE: Aug 24 2007

Nuts - \$10mer azide.fid



d<sub>6</sub>-DMSO  
500.23 MHz

8  
7  
6  
5  
4  
3  
2  
1  
0 PPM

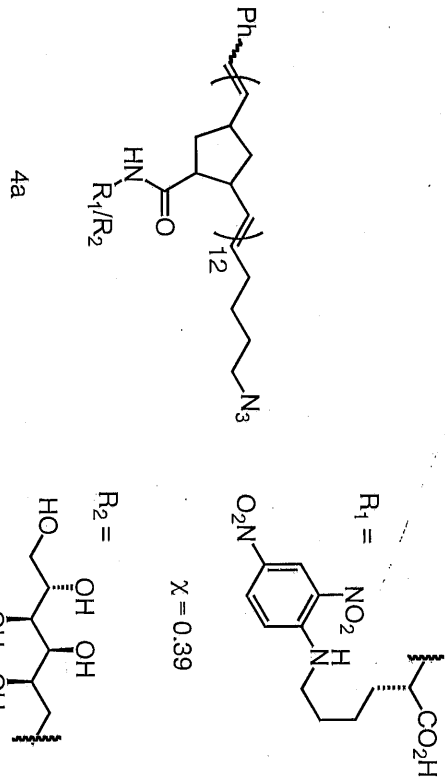


STANDARD PROTON PARAMETERS

F1: 500.231	F2: 125.794	SW1: 8003	PD: 1.0 sec	OP1: 3001.3	PTS1d: 31760 , 32768
EX: s2oull		PW: 4.8 us		NA: 11692	IB: 0.3

USER: -- DATE: Sep 2 2007

Nuts - \$500mer Azide.fid



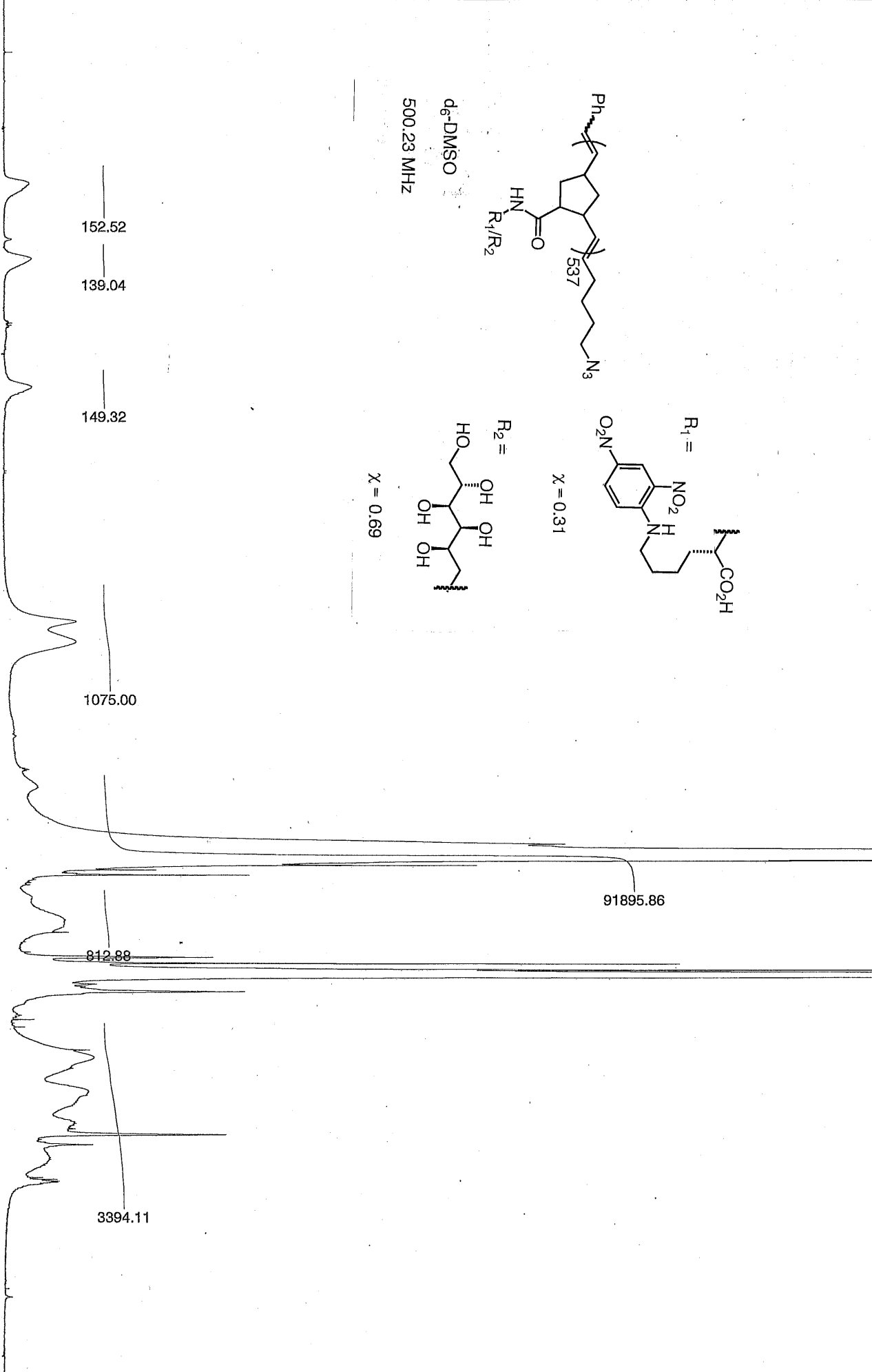
d<sub>6</sub>-DMSO  
500.23 MHz



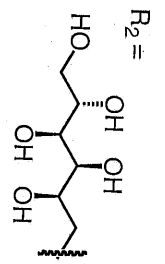
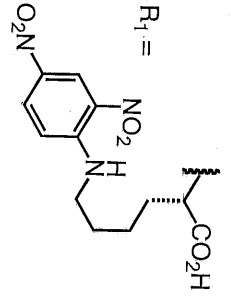
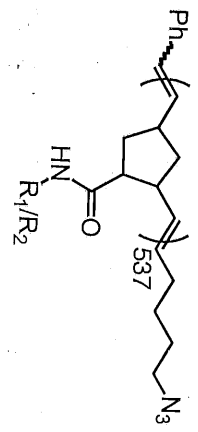
STANDARD PROTON PARAMETERS

F1: 500.231	F2: 125.794	SW1: 8003	PD: 1.0 sec	OF1: 3001.3	IB: 0.3	PTS1d: 31760 , 32768	USER: -- DATE: Oct 25 2007
EX: s2pul		PW: 4.8 us		NA: 8204		Nuts - \$SM-I-130-3-DNP10mer.fid	

10  
8  
6  
4  
2  
0 PPM



$d_6$ -DMSO  
500.23 MHz

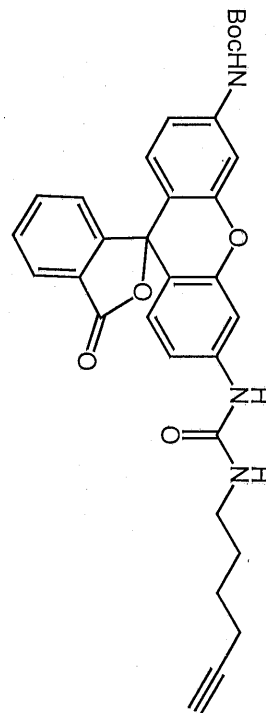


X = 0.69

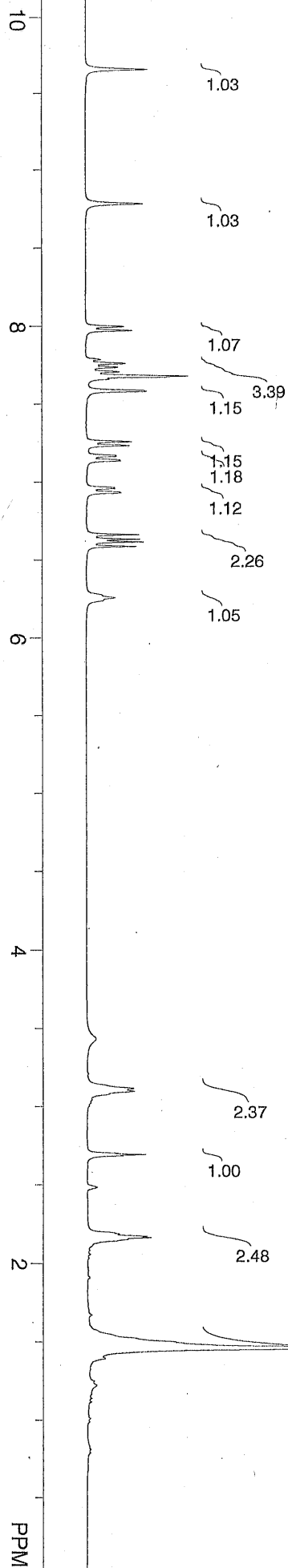
X = 0.31

STANDARD PROTON PARAMETERS

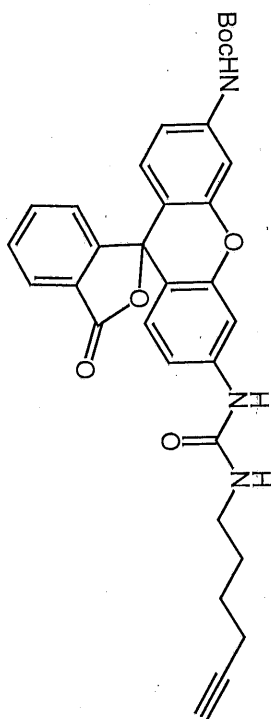
F1: 500.231	F2: 125.794	SW1: 8003	PD: 1.0 sec	OF1: 3001.4	LB: 0.3	PTSID: 31760	USER: -- DATE: Jun 12 2007
EX: s2pul	PW: 4.8 us			NA:		32768	Nuts - \$SM-I-59-500mer-DNP.fid



$d_6$ -DMSO  
299.73 MHz



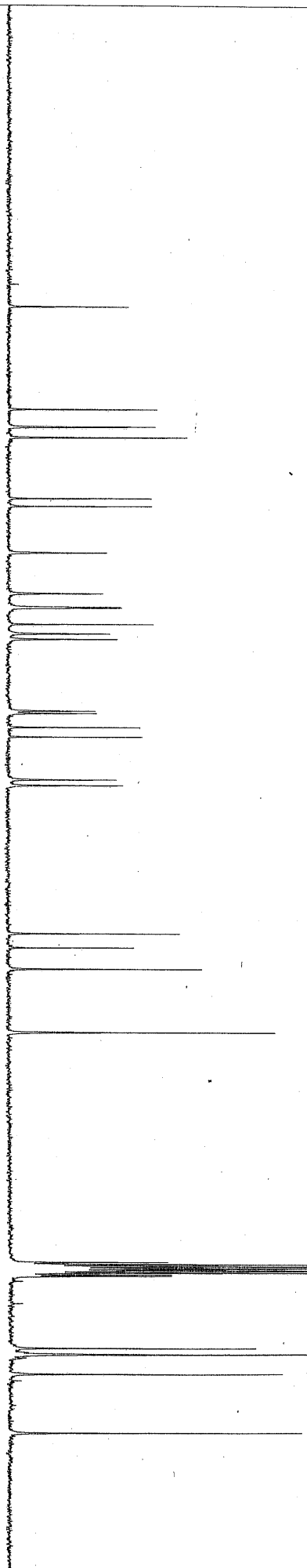
blank line/export/home/walkup/auto\_2007.03.23/mangold\_Fluorophore\_Alkyne\_E  
 USER: -- DATE: Mar 25 2007  
 F1: 299.733 F2: 75.375 SW1: 5995 OF1: 2098.1 PTS1d: 23981 , 32768  
 EX: s2pul PW: 4.9 us PD: 0.1 sec NA: 32 IB: 0.3 Nuts - \$Proton 01.fid



$d_6$ -DMSO

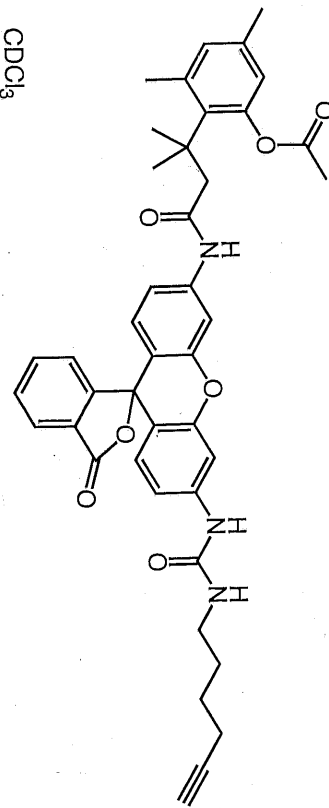
299.73 MHz

200 150 100 50 PPM

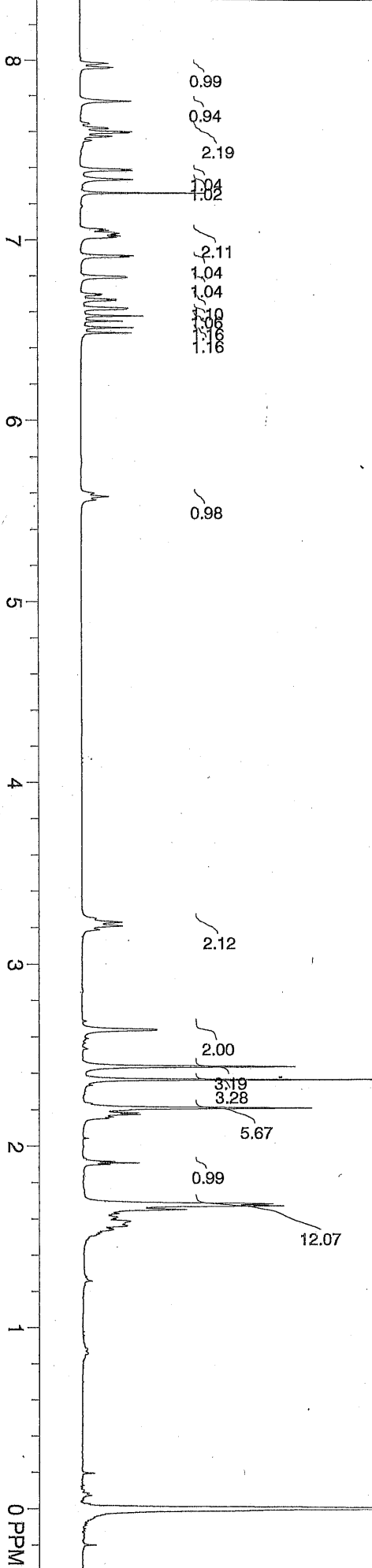


blank line/export/home/walkup/auto\_2007.03.23/mangold\_Fluorophore\_Alkyne\_E  
 F1: 75.376 F2: 299.733 SW1: 1.19608 OF1: 8290.4  
 EX: s2pul PW: 5.8 us PD: 2.0 sec NA: 5000 LB: 2.0  
 PTS1d: 29412 , 32768  
 USER: -- DATE: Mar 25 2007  
 Nuts - \$Carbon 01.fid

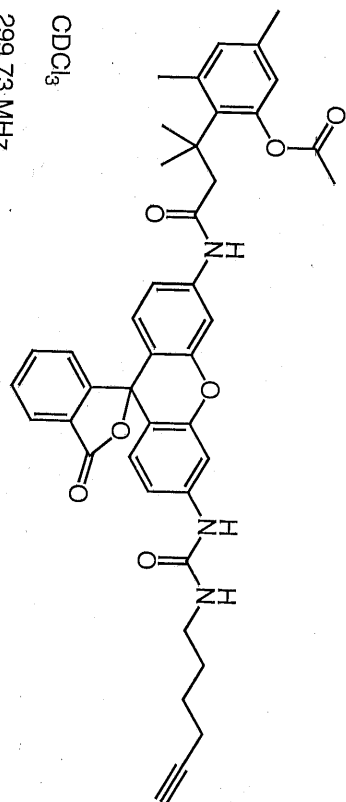




CDCl<sub>3</sub>  
299.73 MHz

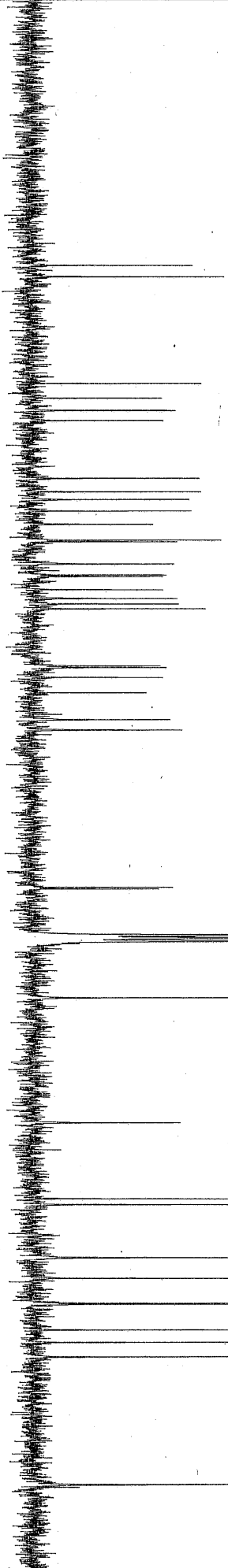


blank line/export/home/walkup/auto\_2007.06.06/mangold\_Trimethyllock\_Flurop  
 FT: 299.732 F2: 75.375 SW1: 5995 PW: 4.9 us PD: 0.1 sec NA: 32 OF1: 2098.5 LB: 0.3 PTS1d: 23981 32768  
 USER: -- DATE: Jun 7 2007  
 Nuts - \$Proton 01.fid

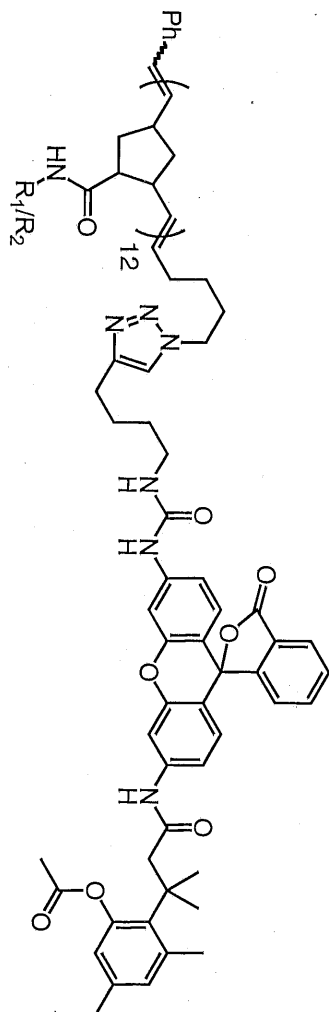


CDCl<sub>3</sub>  
299.73 MHz

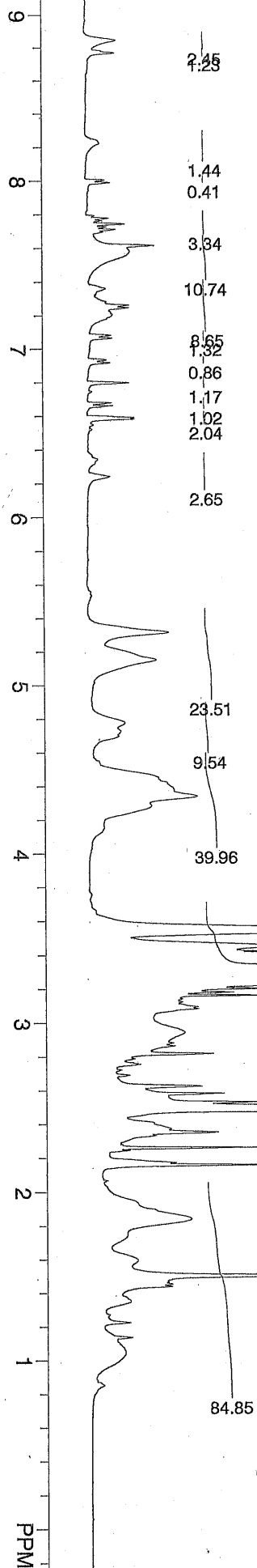
200 150 100 50 0 PPM



Blank line/export/home/walkap/auto\_2007.06.06/mangold\_Trimethyllock\_Flurop  
 F1: 75.375 F2: 299.731 SW1: 19608 OF1: 8275.1  
 EX: s2pul PW: 5.8 us PD: 2.0 sec NA: 2000 IB: 2.0  
 PTSId: 29412 , 32768  
 USER: -- DATE: Jun 7 2007  
 Nuts - \$Carbon 01.fid



d<sub>6</sub>-DMSO  
500.23 MHz

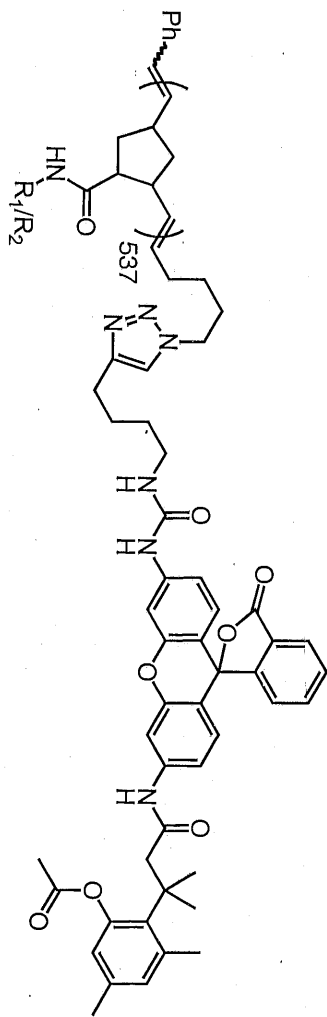


STANDARD PROTON PARAMETERS

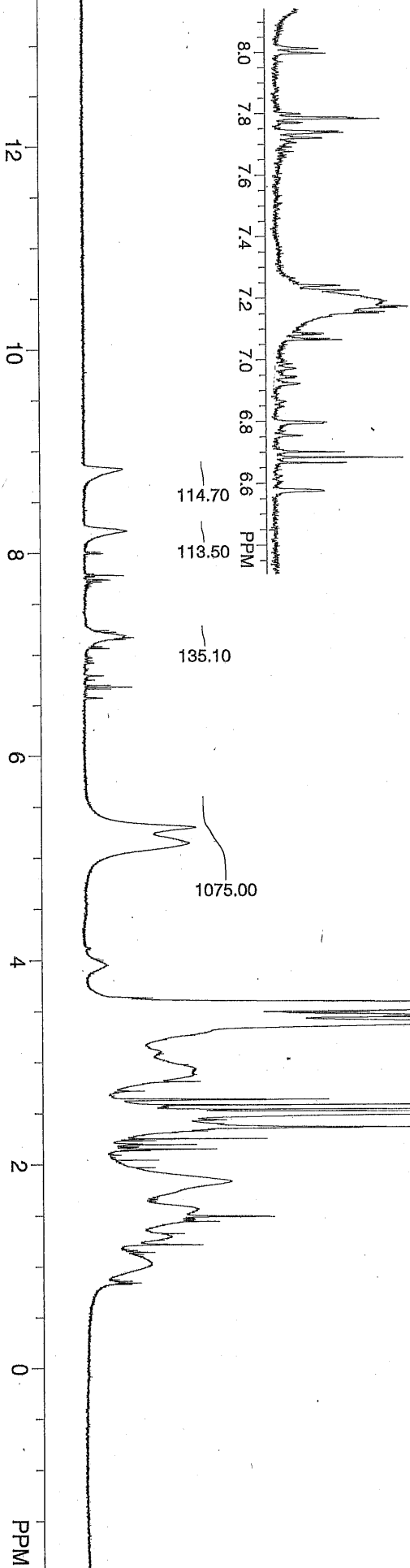
F1: 500.231	F2: 125.794	SW1: 8003	PW: 4.8 us	PD: 1.0 sec	OF1: 3001.3	NA: 11360	LB: 0.3	PTS1d: 31760	32768
EX: s2pul									

USER: -- DATE: Aug 30 2007

Nuts - \$Clicked 10mer.fid



$d_6$ -DMSO  
500.23 MHz



STANDARD PROTON PARAMETERS

F1: 500.231	F2: 125.794	SW1: 8003	PD: 1.0 sec	OF1: 3001.3	LB: 0.3	PTSID: 31760 , 32768	USER: -- DATE: May 22 2008
EX: s2nul		PW: 4.8 us		NA: 800			Nuis - \$clicked500mer.fid